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THE EFFECT ON THE OFFSPRING OF INTOXICATING THE MALE PARENT AND THE TRANSMISSION OF THE DEFECTS TO SUBSEQUENT GENERATIONS

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IT is a thoroughly demonstrated fact that the fertilized egg may be so treated or modified during development as to cause it to give rise to abnormal embryos of definite types. Experiments on the unfertilized egg or female germ cell are not nearly so numerous, are more difficult to perform, and the results are not so decided. The treatment of the male germ cell, or spermatozoon, so as to modify it and to cause a modified development of the egg which it subsequently fertilizes, is an experiment which has rarely been performed with success. In the present communication we wish to consider rather briefly the various methods of treating or modifying the spermatozoon or male germ cell and the result of this modification on the embryo which arises when such a spermatozoon fertilizes an egg. In order to fully appreciate the results obtained by experiments on the sperm it becomes necessary to refer from time to time to the effects derived when the egg is similarly treated.

Since the positive literature bearing on the artificial modification of the sperm is not extensive, I shall first consider it in a general way, and devote the latter part of the paper to the results obtained in a set of experiments

which have been conducted on guinea-pigs for the last few years.

The spermatozoon is more difficult to treat experimentally than is the ovum, on account of the fact that the treatment so often renders it inactive or cripples it in such a way that it is unable to penetrate and to fertilize an egg. The spermatozoon, although apparently delicate, is more or less resistant, so that a mild treatment gives no effect. The difference between the upper non-effective dose of treatment and the fatal or "paralyzing" dose is slight, yet it is the precise treatment between these two points which yields results.

Little has been done in treating chemically the spermatozoa of invertebrates, though some of the hybridization experiments furnish indirect evidence as to what might occur. Herbst's experiment of starting the development of an egg by parthenogenesis and then fertilizing one blastomere with a foreign spermatozoon offers splendid opportunity for investigating the influence of strange substances introduced by the sperm. The spermatozoa of the sea urchin have been subjected to the action of radium emanations by Günther Hertwig, who found that after intensive treatment for several hours such spermatozoa subsequently disturb the processes of division in the eggs which they have fertilized. Paula Hertwig found that fertilized ascaris eggs treated several hours with radium preparations gave pathological division figures; the chromatin bodies showed a tendency to break down and disintegrate; division was slow and the karyokinetic figures were finally completely deranged.

Investigations on vertebrates have been more extensive. The most beautiful results have been obtained on fish and amphibians through the radium experiments of Oscar Hertwig, his son Günther and his daughter Paula. The outcome of these experiments is so striking and is of such serious importance that I shall dwell somewhat upon their significance. About three years ago O. Hertwig published the results of his first radium experiments in

the *Proceedings of the Prussian Academy of Sciences.* At that time he showed that when the unfertilized eggs of a frog are treated for a certain time with radium rays, these eggs, after being fertilized by normal spermatozoa, develop abnormally. Hertwig also found that when the milt or semen of the frog was exposed to the action of radium for a certain time the spermatozoa swimming in it were injured. When eggs were fertilized by these sperm they always developed abnormally. The general type of the abnormalities was the same whether the eggs alone had been treated before fertilization or whether the sperm was treated. When both eggs and sperm were treated the developmental modifications were still more pronounced, though Hertwig claims that the deformities were of a similar type to those which occurred after treating only one of the cells.

Since that time Oscar Hertwig with his son and daughter have extended and analyzed these experiments in a comprehensive fashion. When the sperm of a number of amphibians, frog, toad and salamander, are exposed for five minutes to 5.3 mg. of radium bromide, normal eggs fertilized by such sperm give defective embryos, the defects being generally shown by the central nervous system. They are really of the nature of developmental arrests or degeneration.

If the spermatozoa are exposed for fifteen minutes the effects on development are still more marked. When, however, the sperm in salt solutions are treated intensively for 2 and 3 hours between two mesothorium capsules, the results are most surprising. In one experiment almost all the eggs fertilized by such sperm went *normally,* and in other experiments they went almost normal but slow, yet they were extraordinarily better than eggs that were fertilized by sperm that had been treated for only five minutes. After three weeks the radium larvæ were still behind the control. Hertwig concluded that the spermatozoon had been so injured by the intensive treatment that it could no longer take part in development,

although it could penetrate the egg and cause it to develop parthenogenetically.

A number of important experiments were tried to test the correctness of this conclusion. The most striking of these were those performed by Günther Hertwig in crossing different species.

It is well known that the sperm of the frog will fertilize the egg of the toad or of another species of frog, but the resulting development proceeds for only a short time and the egg usually dies in the blastulæ stage. Günther Hertwig decided that if the influence of the sperm in development was really destroyed by the intensive radium treatments then such a spermatozoon would merely serve as a parthenogenetic agent and the egg should develop in a normal manner, yet be parthenogenetic. He used the eggs of *Bufo vulgaris* (the common toad) and of *Rana veridis* (the green water frog), and the sperm of *Rana fusca*. He ran two sets of each kind of eggs; one set was fertilized by normal *R. fusca* sperm; the other by sperm which had been treated for two or three hours between two capsules of mesothorium. The eggs fertilized by normal sperm did not develop beyond the blastular stage as was expected, while those fertilized with the radiumized sperm developed about normally and hatched from the jelly and gave rise to swimming tadpoles. O. Hertwig repeated this experiment by crossing the eggs of *Triton vulgaris* with the sperm of *Salamandra maculata*. The sperm were treated for $2\frac{1}{4}$ hours between two strong mesothorium preparations. Poll had found that this cross proceeded only as far as the blastular stage and Hertwig confirmed this.

When, however, the semen was treated for $2\frac{1}{4}$ hours a different result was obtained. Many eggs failed to become fertilized, some showed polyspermy, and only six went normally. The chromatin of the sperm was thought to be destroyed and the eggs went by parthenogenesis. Loeb has found in the remarkably wide crosses he has made on invertebrates and vertebrates that the products

of part of the foreign sperm finally act as a poison and cause the eggs to develop abnormally. The types of monsters in these crosses are similar to those produced by treating the eggs with chemical poisons. In many cases these sperm take no part in development but initiate the process by serving as a parthenogenetic agent. Hertwig takes the same position, and further finds, that when the foreign sperm is treated with radium the injurious substance contained in it is killed or destroyed so that the spermatozoon initiates development by parthenogenesis without later causing the development to be abnormal. Bataillon's method of sticking eggs with fine platinum needles to give artificial parthenogenesis is similar, Hertwig thinks, to the use of sperm intensively treated with radium. The treated spermatozoon plays the rôle of the platinum needle in Bataillon's experiment. The male chromatin can no longer combine with the female chromatin, there is no amphimixis. Bataillon, by his sticking method, obtained from 10,000 *R. fusca* eggs only 120 hatched tadpoles, and but three metamorphosed, while in some of Hertwig's radium experiments almost all hatched from the jelly.

The radium experiments of Hertwig give us the first method of artificial parthenogenesis which offers promise for use with mammals. Hertwig suggests that since artificial fertilization is possible in many mammals, one might fertilize with semen which had been intensively treated with radium so that the chromatin was destroyed, and with such sperm artificial parthenogenesis in mammals could be accomplished. Two years before Hertwig made this suggestion Dr. Congdon was trying the effects of radium on the spermatozoa of mice and rats in the anatomical laboratory at Cornell and is now continuing these experiments in the anatomical laboratory at Stanford University; up to now he has not succeeded in obtaining fertilization with the modified spermatozoa, though of course much experimentation is necessary in order to establish the proper intensity of the treatment.

These experiments of Hertwig also afford interesting data as to the nature and importance of the part played by the chromatin in development. The cells of the embryos which resulted from eggs fertilized by intensively treated sperm were found by O. Hertwig, P. Hertwig, and Poll, to contain the reduced number of chromosomes showing that the paternal chromatin had been destroyed by the treatment. Günther Hertwig found the nucleus volume in radium larvæ to be one half the size of the nucleus in the control; he measured the mass of nuclei of nerve cells, liver cells, blood corpuscles, embryonic muscle cells, etc. The entire larva was smaller. P. Hertwig found the male pronucleus derived from intensively treated sperm to be modified in the first and second divisions of the frog's egg and Opperman found the same in the trout. O. Hertwig found in *Triton* eggs that the intensively radiumized male chromatin took no part in the developmental process and the soma cells contained one half the chromosome number. The male chromosome set falls out of the development and the soma nuclei contain only the female set.

Finally, Hertwig obtained another most striking result which may be mentioned, although it is not entirely in line with the present subject. When eggs instead of the spermatozoa were subjected to intensive treatments of 2 to 5 hours with radium, the chromatin of the female pronucleus was found to be broken down and destroyed. If eggs, after such intensive treatment, were fertilized by normal sperm, it was found that they developed almost normally, although when eggs were treated from 15 minutes to $\frac{1}{2}$ hour they always developed abnormally though fertilized with normal spermatozoa. Hertwig, therefore, concludes that the intensively treated eggs fertilized by normal sperm develop by the process of merogony; that is, the egg nucleus being destroyed by the treatment, the sperm nucleus enters the egg and causes development to proceed in the same way that the female pronucleus acts in parthenogenesis. Only one set of chro-

mosomes, either the paternal or maternal, is necessary for development of the egg.

During the summer of 1912 I treated the spermatozoa of fish with various salts and organic substances with negative results. When the treatment was sufficiently strong to affect the spermatozoa it rendered them incapable of fertilizing the eggs. A method could no doubt be devised for modifying fish spermatozoa with various chemicals and of course radium does modify the fish sperm as Opperman found.

Only a few experiments have been performed in attempting to modify the offspring of birds by injuring the male. Todde found that the offspring from alcoholized roosters were not quite normal and that the roosters did not succeed as well as usual in fertilizing eggs. Lustig's experiments showed that by inoculating fowls with abrin the offspring were rendered less resistant to inoculations of abrin than were control animals of the same age. This result followed the inoculations of either parent, the male as well as the female.

A more extensive literature bears upon the actions of poisons on the male germ cells of mammals, though most of the cases are uncontrolled observations. The treatment of the germ cells of mammals is a more complex proposition than the experiments on those lower forms in which the fertilization is external and where, for this reason, the eggs and spermatozoa may be treated directly. In mammals the stimulus must be applied through the animal body and the case is thus complicated since it is often impossible to differentiate between the direct action of the substance applied and the secondary effects due to the responses of the parental body to the treatment. With certain treatments, however, the case is not so complex as would appear at first sight, since the substances may pass into the blood stream and the lymph and act directly on the germ cells just as they do on other tissues and cells of the body.

In experiments to modify the germ cells of mammals

the first proposition becomes then, to determine whether the substances used reach the germ cells directly. One of the best substances for such experiments is alcohol, since its action and distribution in the body has been largely studied and since it acts so decidedly to modify the developmental processes, as many workers have found on invertebrates, and as I have shown by treating fish eggs with this substance.

It is a well known and generally accepted fact that alcohol does cause changes and degeneration in many of the tissues of animals and man. The question arises, how, then, can the reproductive tissues, the ova and spermatozoa escape? Nicloux and Renault have found that alcohol has a decided affinity for the reproductive glands. In the testicular tissues and the seminal fluid an amount of alcohol is soon present which almost equals that in the blood of an individual having recently taken alcohol. The proportion of alcohol in the testis as compared with that in the blood was as 2 to 3, and in the ovary of female mammals as 3 to 5. From these observations it must follow that alcohol may act directly on the ripe spermatozoon shortly before it fertilizes the egg, and if this substance injuriously affects the germ cells, then one should expect to find an indication of the injury in the resulting development as Hertwig has found from his radium treated spermatozoa.

There are a number of observations on human beings bearing on this point, though they probably all need confirmation by experimentation on lower mammals. Lippich claims to have observed 97 children resulting from conception during intoxication. Only 14 of these were without noticeable defects. Twenty-eight were scrofulous, three had "weak lungs," three showed different atrophic conditions, one watery brain, four feeble-minded, etc. Sullivan reported seven fairly authentic cases of drunkenness during conception; six of the offspring died in convulsions after a few months, and the seventh was stillborn.

Rösch was the first, in 1837, to study the reproductive glands of alcoholics and found degeneration of the testicles. Lancereaux described a parenchymatous degeneration of the seminal canals. Simmonds (1898) found azoospermie (spermatozoa without tails) in 60 per cent. of cases of chronic alcoholism; 5 per cent. of these men were sterile. Kyrle reported three cases of total atrophy of the testicular parenchyma in which death had resulted from cirrhosis of the liver due to alcohol. He attributed the atrophy of the testicle to the cirrhosis of the liver and not to chronic alcoholism.

Bertholet (1909) has made an extensive examination of the influence of alcohol on the histological structure of the germ glands, particularly on the testicles of chronic alcoholics. He found testicular atrophy in alcoholics with no cirrhosis of the liver. Bertholet observed partial atrophy of the testicles in the majority of 75 chronic alcoholics. The men died between the ages of 24 and 57 years, the greatest mortality being between 30 and 50 years. In 37 cases, excluding syphilitics, a microscopical examination showed a more or less diffuse atrophy of the testicular parenchyma and a sclerosis of the interstitial connective tissue. The canals were reduced in size and their lumina obliterated. Spermatogonia were atrophic. It was generally impossible to differentiate spermatocytes or spermatids. There were no dividing cells and no spermatozoa. These conditions with slight variations were found in 24 cases. Such atrophic structures were present in one drinker only 29 years old. In 4 cases of cirrhosis of the liver the testicular atrophy had not progressed very far and spermatozoa were still present.

The extreme conditions of atrophy of the testicles were only found in alcoholics. Observing the testicles of non-alcoholics that had died of various chronic illnesses, such as tuberculosis, no atrophy of the testicles or thickening of the membrana propria was found. Two old men of 70 and 91 years still possessed spermatozoa in the canals.

Bertholet concluded that the atrophy he observed was not due to old age, cirrhosis of the liver, or other systemic conditions, but to the effects of chronic alcoholism on the reproductive glands. Weichselbaum has confirmed the observations of Bertholet.

It is certain, however, that the chronic alcoholic is not so often rendered sterile as Bertholet's study would lead one to believe. It is not rare to find alcoholics with large families. My experiments on mammals may not be of sufficient duration at the present time, yet I have male guinea-pigs that have been almost intoxicated on alcohol once per day for six days a week for a period of 32 months, which are still good breeders. Thirty-two months of a guinea-pig's existence is proportionately equal to a good fraction of a human life. A number of these animals have been killed and their testicles examined microscopically and found to be normal. In some cases where the male had failed to succeed in impregnating the female for several times, one of his testicles was removed and studied microscopically; the testicle was found to be normal and the male later gave offspring by other females. Ovaries have been similarly examined and in no case has the alcoholic treatment caused a visible structural change in the reproductive glands. The actual physiological proof of the efficiency of the organs is shown by the ability of the animals to reproduce. Although there is no visible structural change in the germ cells, nevertheless, they have been modified by the treatment to an extent sufficient to cause them in most cases to give rise to defective embryos or weakened individuals which die soon after birth.

Nicloux has carefully demonstrated on dogs and guinea-pigs the passage of alcohol from the blood of the mother into the tissues of the embryo. After a short time the amount of alcohol in the blood of the fetus is about equal to that in the blood of the mother, while there is really slightly more alcohol in a given weight of the tissues of the fetus than is to be found in an equal

weight of liver tissue from the mother. The reality of the passage of alcohol from the mother to the fetus demonstrates the possibility of the intoxication of the fetus.

There is an abundance of data bearing on the effects of parental poisoning on the human offspring, yet almost all of it is complicated. The question arises whether the defects of the offspring are actually due directly to the parental poisoning or to the often degenerate condition of the parent. With lower mammals this question may be controlled, since vigorous individuals with no physical weaknesses may be selected for study. One of the most interesting human cases is that Forel cites as recorded by Schweighofer. A normal woman married a normal man and had three sound children. The husband died and the woman married a drunkard and gave birth to three other children; one of these became a drunkard; one had infantilism, while the third was a social degenerate and drunkard. The first two of these children contracted tuberculosis, which had never before been in the family. The woman married a third time and by this sober husband again produced sound children. This is a logical experiment, the female was first tested with a normal male and gave normal children; when mated with an alcoholic male the progeny were defective. She was later tested again with a normal male and found to be capable of producing sound offspring. A number of such cases are on record but all are open to the question whether the defective offspring are actually due to the effects of the poison on the parent, or to the fact that the parent may have been weak and degenerate from the beginning.

Other substances than alcohol seem to act directly on the germ cells of man and mammals, and these actions are more important since there is no reason to believe, for some of them at any rate, that they accompany a degenerate condition. Constantine Paul long ago pointed out that the children of lead workers were often defective.

He made the interesting observation that when the father alone was employed in such work his children were affected. In 32 conceptions with such fathers 12 resulted in premature labor and stillbirths, 20 living births occurred but only 3 children survived. Eight died the first year, 4 the second, and 5 the third year.

Mairet and Cambemale in 1888 were the first to experiment on the influence of alcohol on the mammalian offspring. They treated a dog for 8 months with absinthe (11 gr. per day per kilo of animal weight) and paired this alcoholized dog with a normal bitch. Twelve young resulted; 2 were born dead, 3 died within 14 days, and the others died between 32 and 67 days of intestinal catarrh, tuberculosis, etc. In a second experiment, both parents were mated while normal, then the female was treated for 23 days (2.75 to 5 gr. of absinthe of 72 per cent. per day per kilo). Of 6 young 3 were stillborn, 2 had normal bodies though of weak intelligence, while one was very sluggish. The evident criticism against this experiment is that an insufficient number of animals was used and there was no control. It is very difficult to rear pups in a laboratory; when apparently perfectly normal, they often die shortly after birth.

Hodge, in 1897, obtained similar results. From one pair of alcoholic dogs he observed 23 pups, 8 were deformed, 9 were born dead, while only 4 lived. In a control set, 41 individuals lived, 4 were deformed, but there were no stillbirths.

Nice has recently published results of treating mice with alcohol. He finds little, if any, effect of the treatment on the offspring. Considering his method of administering the alcohol and the results obtained, the doses used were probably insufficient to produce effects. It may also be possible that mice are more resistant to alcohol than are other mammals. I have discussed these experiments in a previous communication.

Experiments

Three years ago a series of experiments were begun on guinea-pigs with the hope of modifying the type of embryo in mammals so as to produce definite monstrosities as one is able to do with lower vertebrates. This primary object has not been fully accomplished, yet the experiments have demonstrated several significant points and have shown that an alcoholized male guinea-pig almost invariably begets a defective offspring even when bred to a vigorous normal female.

Normal, healthy animals are selected for the experiment, and in all cases they are first tested by a normal mating in order to establish their ability to produce vigorous offspring. After such a test the treatments are begun. During the experiments the treated males and females are mated from time to time with normal animals, and in addition, control matings of normal individuals are made. Some of the specimens are treated with alcohol and ether. These substances were used since they readily act upon animal cells and since I had studied their effects on the development of fish embryos and found them to cause rather definite and easily recognizable defects in the central nervous system and organs of special sense.

Method and Technique

In the beginning of the experiments alcohol was given along with the food, but the animals ate less and the food did not apparently agree with them. It was then administered in dilute form by a stomach tube; this method disturbed digestion and seemed to upset the animals considerably. It is certain that alcohol given to animals through the stomach deranges their digestion and appetite to such an extent that the experimenter is unable to determine whether the resulting effects are due to the alcohol, as such, or to the general deranged condition of the animal. When given in the drinking water they take little or none of the water and the treat-

ment is insufficient. For these reasons an inhalation method of treatment has been resorted to which, as far as experience goes, has no serious disadvantages and does not complicate the conditions of the experiment.

A fume tank of copper is made of sufficient size to supply breathing space for 4 or 5 guinea-pigs at one time. The tank is arranged with four outlets, so that definite amounts of the fumes may be passed through in a given time and the ventilation controlled. In this way each animal could be given about the same amount of the substances. The individuals, however, differ so in their resistance to the treatment that it has been found better to treat all to about the same degree of intoxication. This physiological index is more reliable as each animal is thus affected in a similar fashion each day. For this purpose they are placed in the fume tank on a wire screen, and absorbent cotton soaked with alcohol is placed beneath the screen, and the animals inhale the fumes. The tank was described and illustrated in a previous article.

Ether is given in a similar manner, except that the animals are much more readily overcome and must be carefully watched while inhaling even the most dilute doses.

In order to avoid handling the females during late pregnancy, a special treating cage is devised. An ordinary box-run with a covered nest in which the animal lives is connected by a drop-door with a metal-lined tank, having a similar screen arrangement to that of the general treatment tank. The pregnant animal may be driven daily into the tank and thus treated with alcohol fumes throughout her pregnancy without being handled in any way that might disturb the developing fetus.

DIRECT EFFECTS OF THE TREATMENT ON THE ANIMALS

Many of the animals have now been treated almost to the point of intoxication for six days per week for nearly three years. They are affected by the alcohol fumes in

different ways; certain ones become drowsy and stupid, while others become excited and sometimes vicious during the treatment, constantly fighting and biting at others in the tank. One male always had to be treated alone on this account. The fumes are inhaled into the lungs and pass directly into the circulation, so that the animals show signs of intoxication very soon after being put into the tank, yet the intake of alcohol is so gradual that they may remain for one hour or more without becoming totally anesthetized. The mucosa of the respiratory tract is considerably irritated during the first few days or weeks of the treatment, but later becomes hardened and little effect can be noticed. The cornea of the eye is greatly irritated and often becomes milky white and opaque during the first few months; but later this clears up in most of the specimens and the animal is able to see perfectly, though one male that has been treated for 32 months is now entirely blind. The general condition of the animals under the treatment is very good; they all continue to grow if treated before reaching their full size, and become fat and vigorous, taking plenty of food and behaving in a normal manner in every particular.

Certain of the animals have been killed at different times during the experiment and their organs and tissues studied microscopically; all have seemed entirely normal. The tissues of one female were examined after she had been treated for over a year, and the heart, stomach, lungs, liver, kidney, etc., were all normal. She was generally fat but there was no fatty accumulation in the parenchyma of any of the organs except possibly a slight excess in the adrenal glands.

As mentioned above several of the animals, both males and females, have been partially castrated during the experiments and the ovaries and testis have been found to be in healthy condition.

The treated animals are, therefore, little changed or injured so far as their behavior and structure goes. Nevertheless, the effects of the treatment are most

decidedly indicated by the type of offspring to which they give rise, whether they are mated together or with normal individuals.

EFFECTS ON THE OFFSPRING

The animals have been mated in various combinations. First, treated males have been paired with normal females, the paternal test; this is the crucial test of the influence of the treatment on the germ cells. In this case the chemically modified or weakened spermatozoon can alone be responsible for the defective offspring, since the egg is normal and develops in a normal environment in the healthy mother.

Second, treated females are paired with normal males, the maternal test. This combination offers two chances for injuring the offspring. Either the ovum may be defective as the result of the treatment which the mother has undergone, and may thus give rise to a defective individual; or secondly, the developing embryo may be affected directly by the alcohol in the system of the mother, since Nicloux has shown that this substance may pass from the blood of the mother into the tissues of the fetus. Thus the intoxication of the embryo may modify its forming structures in the same way that a fish embryo develops deformed organs and parts when in sea-water to which alcohol has been added.

The third combination is the mating of two alcoholic individuals. This is the most severe test and offers the greatest chance for defective offspring.

Before the experiment or treatment begins the guinea-pigs are all tested by normal matings and are found to give normal vigorous offspring. They continue to give normal offspring until the treatment has lasted for some time. The effect accumulates slowly and is not noticed at once. A number of experiments in which the treatment of a female was commenced at the beginning of pregnancy have so far given rather indefinite results, although a slight effect may be indicated.

In all 124 matings of treated individuals have been made. One hundred and three of these have reached full-term and are recorded. Twenty-one matings are not yet due. From the 103 full-term matings only 52 young have survived and most of these are somewhat under size and show their affected condition in the type of offspring to which they give rise. Yet their parents were all unusually large and originally strong animals.

From 35 control matings 56 healthy offspring have been derived which continue to produce normal animals in the following generations, in a few cases now to the fourth generation.

A tabulated summary of the results may be arranged as indicated in Table 1. The conditions of the animals in the mating pairs are shown in the first column of the table and the total results of the matings are indicated in the following columns.

The first horizontal line gives the record when alcoholic males are paired with normal females. Fifty-nine such matings have reached term, 25 of these gave negative results or early abortions. Some embryos were aborted during very early stages and were generally in such poor condition when found in the cages that little could be learned from them. They were partially or completely eaten by the mother in most cases. The males were always kept with the females during favorable periods for a number of days, usually about three weeks, and conception should have occurred in all cases, as it did with the control matings.

Thirty-four of the 59 matings resulted in conceptions which ran the full term. Eight, or about 24 per cent. of these, were stillborn litters, consisting in all of 15 individuals. Most of these were somewhat premature; in a few cases their eyelids were still closed and the hair was sparse on their bodies. (A normal guinea-pig at birth is well covered with a hairy coat, its eyelids are open and it very quickly begins to run about.)

Twenty-six, or only 44 per cent., of the matings pro-

duced litters of living young. These litters contained in all 54 individuals. Twenty-one, or almost 40 per cent., of these young guinea-pigs died within a few days or less than four weeks after birth, while 33 of them survived. *Thus, out of 69 full term young, of which 54 were born alive, only 33 have survived, and many of these are small and excitable animals, and although not treated themselves have since given rise to defective offspring in several cases where they have been mated with one another.* On the other hand, 35 control matings have produced 32 living litters consisting of 60 individuals, only 4 of which have died and 56 are perfectly normal animals.

It is of interest that the young animals before dying show various nervous disturbances, having epileptic-like seizures, and in most cases die in a state of convulsion.

The important fact in the above case is that the father only was alcoholic, the mother being a normally vigorous animal. *This experiment clearly demonstrates that the paternal germ cells may be modified by chemical treatment to such a degree that the treated male will beget abnormal offspring even though he be mated with a vigorous female.* A reconsideration of the figures in the first line of the table shows really how decidedly the injured spermatozoon expresses itself in the fate of the egg with which it combines.

For comparison the second line of the table shows the results of matings between alcoholized females and normal males. These matings might be expected to give more marked results than the previous ones, since in the treated female not only the germ cells may be affected, but the developing embryo itself may be injured by the presence of alcohol in the blood of the mother.

There are 15 matings between alcoholized females and normal males. Three of these, or 20 per cent., gave negative results, or were possibly aborted very early. Three stillborn litters were produced consisting of nine individuals, while 60 per cent. of the matings gave living litters. This result is better by 16 per cent. than that

obtained when alcoholized males were paired with normal females. The proportion of surviving individuals is, however, less from the treated females than from the treated males. The 9 living litters contained 19 young, 9 of which died soon after birth and 10 survived. Thus out of 28 full term young only 10, or about 36 per cent., survived, while 64 per cent. of the offspring were lost; in the above cases where the male alone was alcoholic almost 48 per cent. of the full term young survived.

TABLE I

CONDITION OF THE OFFSPRING FROM GUINEA-PIGS TREATED WITH ALCOHOL

Condition of the Animals	Number of Matings	Negative Result or Early Abortion	Stillborn Litters	Number Stillborn Young	Living Litters	Young Dying Soon After Birth	Surviving Young
Alcoholic ♂ by normal ♀	59	25	8	15	26	21	33
Normal ♂ by alcoholic ♀	15	3	3	9	9	9	10
Alcoholic ♂ by alcoholic ♀ ..	29	15	3	6	11	7	9
SUMMARY.....	103	43	14	30	46	37	52
Normal ♂ by normal ♀	35	2	1	4	32	4	56
2d generation by normal.....	3	0	0	0	3	0	4
2d generation by alcoholic....	3	0	2	5 1 def.	1	0	2
2d generation by 2d generation	19	7	0	0	12	6 1 def.	13
Female treated during pregnancy.....	4	0	0	0	4	1	7

The third horizontal line indicates the results of pairing alcoholized males with alcoholized females. The effects of the treatment in this case are slightly more marked than in either of the above lines. Twenty-nine such matings gave in 15, or more than 50 per cent., of the cases negative results or early abortions. Three stillborn litters occurred, each consisting of two individuals. Only 11 living litters were produced. These contained 16 young, 9 of which survived while 7 died soon after birth.

A comparison of this combination with the control matings given in the fifth line shows in a decisive manner the really detrimental effects of the treatment. In the one case only 9 surviving young were obtained from 29

matings, while in the other the control animals gave 56 surviving young from 35 matings.

The fourth line summarizes the results of the matings made with treated individuals. A total of 103 matings have run the full term; 43, or almost 42 per cent. of these, have given negative results or early abortions; while 35 control matings failed in only two cases to yield a full term litter. Fourteen, or 13½ per cent., of the matings gave stillborn litters consisting of 30 individuals. Only one stillborn litter occurred in the 35 control matings; this was a large litter of 4 young and the mother seemed almost unable to carry them. The 103 matings of treated animals gave only 46 living litters, about 45 per cent., while 32 living litters, or 91½ per cent., were produced by the 35 control matings. The 46 living litters from the alcoholic individuals contained 89 young, 37 of which died shortly after birth and 52 survived. The 32 living litters from the normal animals consisted of 60 individuals, only 4 of which died while 56, or 93 per cent., of these survived.

Of 119 full term young, 30 of which were stillborn, produced by the alcoholic animals, only 52, or less than 44 per cent., survived as against the 56, or 87½ per cent., survivors among the 64 full-term control offspring.

The bottom line of the table shows that 4 normally mated females treated with alcohol during the period of gestation gave 4 living litters, consisting each of 2 young. One out of the 8 young died soon after birth. These few cases would seem to indicate that the treatment, when started at the beginning of gestation, was not particularly injurious to the embryos developing *in utero*.

ARE THE EFFECTS ON THE OFFSPRING TRANSMITTED?

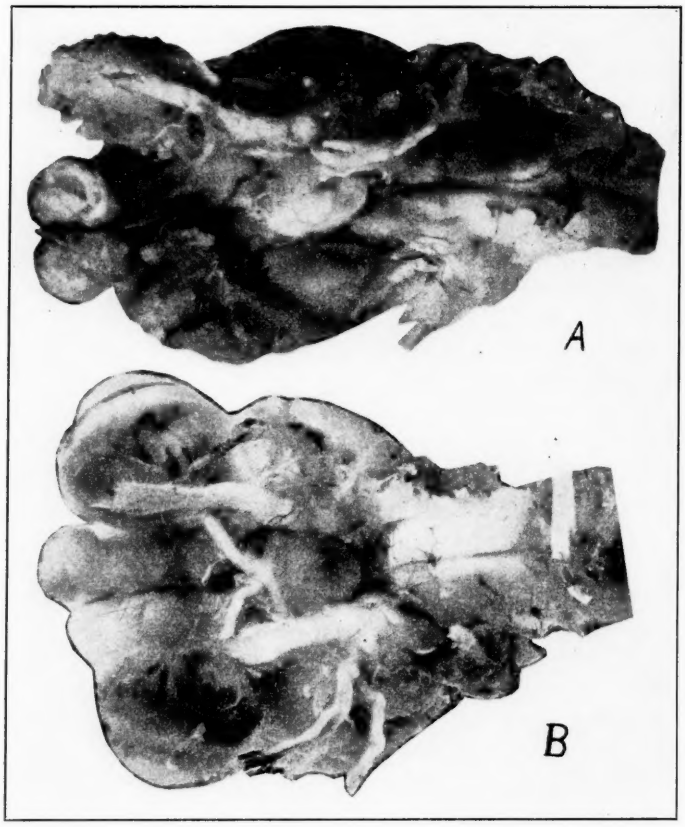
The offspring derived from the alcoholic individuals are termed second generation animals, and were not treated with alcohol. The sixth, seventh and eighth lines of the table represent the data obtained from the few full time matings that have been made with these guinea-pigs.

In three cases second generation animals have been mated with normal individuals and have produced perfect results, though the litters have been small. Three litters are recorded containing a total of 4 young, all of which survived. It might seem as though the normal mate counteracted any defect which may have been present in the second generation animals.

The mating of second generation individuals with alcoholized guinea-pigs gave decidedly different results. The seventh line shows that 2 out of 3 such matings produced stillborn young. In one of these cases the female was of the second generation and the male alcoholic, and in the other case the reverse condition existed; yet both combinations gave dead offspring, one litter of two and one of three individuals. One of these specimens from the second generation female by the alcoholic male was grossly deformed. The third mating gave two surviving young. At present there are too few matings of this combination from which to draw conclusions, yet the results obtained are the most disastrous of all.

Nineteen matings between second generation animals have been made. The outcome in these cases compares very unfavorably with that from the control matings, while the data are much of the same type as those obtained from the alcoholic combinations. Seven, or almost 37 per cent., of the matings gave negative results. Twelve living litters were born consisting of 19 individuals, 6, or about 32 per cent., of which died very soon after birth and showed various nervous disorders. One was entirely eyeless and decidedly deformed.

From the number of records available one might conclude that the effects of the alcoholic treatment were as pronounced upon the offspring of the second generation animals, although they had not been directly treated, as they were upon the offspring of alcoholized individuals. The poison acts upon the cells and tissues of the body, the germ cells as well as other cells, and an offspring derived from the weakened or affected germ cell has all



EXPLANATION OF PLATE

Ventral surfaces of two guinea-pig brains. *A*, the brain of an animal which was entirely eyeless, no optic nerves or tracts are present. *B*, the brain from another member of the same litter. This animal was defective and died shortly after birth, but possessed normal eyes as the photograph shows and the optic nerves are clearly seen passing medially to form the chiasma and from there the optic tracts are shown along the anterior margins of the tuber cinereia. All of these optic structures are missing in the brain above.

the cells of its body, both soma and germ, defective since each of the cells is a descendant of the injured germ cell combination.

These are the initial experiments with mammals to show *that an injury of the germ cells may express its effects on the offspring and be passed through subsequent generations.*

The actual outcome of the experiments may be more fully recognized by a consideration of one of the most striking cases. A large normal female, weighing about 700 grams, had given two normal young by a control mating and had since given non-viable young by a mating with an alcoholic male. She was then mated with another large strong alcoholic male which weighed 740 grams and which had given before this mating apparently healthy offspring by normal females. The mating resulted in the production of 4 young, all small and rather excitable in their behavior. These individuals from the normal mother and the alcoholized father grew slowly, although they ate freely and appeared to be well. They remained small and below the average in weight. Three were males and one was a female.

One of the males was mated with a normal female and two normal young resulted. He was then mated with a female from an alcoholic father and she gave birth to two small young; one of these offspring was only half size and very excitable. He was then mated with a female from an alcoholic mother and one small young was produced.

A second one of the three males was mated with a normal female which produced one large apparently normal offspring. He was then mated with a female from an alcoholic father and two small young resulted, one of which died within five days and the other is weak and nervous. He was again mated with a normal female and one normal young was produced.

The third male was mated with his sister and she gave birth to 3 young. One of the young died when one day

old, having been in a constant tremor since its birth; another lived for nine days but whenever it attempted to walk it was seized with spasmodic contractions; the third specimen exhibited the same nervous manifestations and was completely eyeless. This animal died eight days after birth and an examination of the brain showed an entire absence of optic tracts, as may be seen in Plate 1*A*.

In the development of this animal it is probable that the optic vesicles were suppressed and never arose from the brain. Thus, no eyes, optic nerves, or optic tracts could have formed. This particular eyeless condition in these experiments is of interest since one is readily able to suppress the origin of the optic vesicles in fish and chick embryos by similarly weakening the embryo with treatments of alcohol, ether, etc.

The mother of these offspring was remated with her brother, but she died six weeks later, not becoming pregnant. She was in an emaciated condition but had always been less than half normal weight.

The three extremely weak and defective offspring were doubtless due to the fact that both of their parents had similarly weakened or injured constitutions, having resulted from a single mating of a normal female with an alcoholized male. The eyeless offspring and the other two nervous non-viable individuals should not be interpreted as due merely to the fact that their parents were brother and sister. Several normal matings of brother and sister have been made during the experiment and perfectly healthy offspring have been produced. In the studies of heredity conducted on guinea-pigs brother and sister are crossed with impunity, in no way weakening their offspring. The significant point in the present consideration is that the two animals coming from the same mother and treated father may have had similar weaknesses or defects and the combination of two such individuals resulted in offspring which exhibited these defects to a more decided extent. The three animals were far more defective than their parents and owed their defects

to the modified condition of the germ cells of the grandfather from which they descended.

Consideration of Individual Matings and Responses

Studying the data of individual animals, another point of some importance presents itself. This is the fact that the same male often yields very different results when bred to different females, although the females are similar so far as the experiment goes, being either all normal or alcoholic. This may be due to the varying degrees of resistance or hardiness possessed by the germ cells of different individuals. A part of this difference may be due to the fact that as the treatment advances the germ cells are more affected and the results do actually become more pronounced.

Male No. 5 that has been treated with alcohol for two and one half years, has been mated 25 times. Eleven of these matings have yielded negative results; 4 were with alcoholic females and 7 were with normal. Three of the matings gave stillbirths, 2 with normal females and one by an alcoholic female. Only 11 of the 25 matings gave living litters, 4 of these were non-viable and 7 litters survived. Five of the 7 surviving litters were from normal females and 2 from alcoholic mothers. *This alcoholic male has, therefore, begotten only 7 surviving litters out of the 14 full term litters born and out of a total of 25 matings.*

Alcoholic male No. 6 shows a still worse record. Out of a total of 21 matings, 10 have given negative results, 5 by normal and 5 by alcoholic females. Two matings gave stillborn litters, one from a normal female and one from a second generation female. Nine living litters resulted from the 21 matings, 3 from alcoholic females and 6 from normal. Five of the nine litters born alive were non-viable, the young dying soon after birth. *Only 4 litters survived out of the 11 reaching term and out of the total of 21 matings.* Three of these surviving litters were from normal females and one was from an alcoholic mother.

Alcoholic male No. 43 has been mated 10 times. Three of the matings gave negative results, two by alcoholic females and one by a normal. One stillborn litter resulted from a mating with an alcoholic female. Six living litters came from the 10 matings. The young in 3 of these litters died soon after birth and 3 litters survived.

Alcoholic male No. 45 has now been treated about one and one half years and has been mated 9 times. One mating with a normal female gave a negative result. One mating with a normal female gave a stillborn litter. Seven living litters were produced, 5 of which survived, 2 from alcoholic females and 3 from normal females. The data in this case appear slightly better than in the foregoing but this is due to the fact that only a few matings have been made and most of these during the early stages of the treatment, when the effects are not so pronounced.

All of the other alcoholized males in the experiments show comparable records.

A reference to Table I shows that with three exceptions 35 matings of normal males with normal females gave living litters containing in all 60 individuals, only 4 of which failed to survive. This record stands in striking contrast to the data recorded above from the 4 alcoholic males, and it shows convincingly that the alcoholic treatment has affected the germ cells of these males so that they are no longer capable of producing entirely normal offspring even though they be mated with normal females.

The outcome of the successive matings of fifteen different females is tabulated in Table II. The varying ways in which the same individual has responded in different matings is noticeable. Number 15, a normal female, shows an instructive record. Mated with alcoholic male No. 6 she gave two stillborn young; mated with alcoholic male No. 5 a negative result; remated with No. 5, two young were born and both died of convulsions during the fourth week; then mated with a normal male a normal vigorous offspring was produced; mated again with alco-

holic male No. 5 an apparently normal guinea-pig was born; mated with No. 43, an alcoholic male, she gave 2 young which only lived for two days; then mated again with a normal male she produced 2 vigorous offspring and finally mated with No. 69, an etherized male, 2 young were born, one of which died at birth. Thus, out of the 8 matings, 2 with normal males gave perfectly normal offspring, while 5 out of the 6 matings with treated males gave disastrous results and only one of these matings resulted in the production of an apparently normal young.

Number 56, a normal female, mated to a normal male, No. 48, gave 2 normal young; with alcoholic male No. 45 gave 3 premature stillborn fetuses; again to a normal male No. 80 gave 3 normal offspring; and finally again to alcoholic male No. 45 she gave one very small young.

Normal female No. 63 gave two normal individuals by a normal mating and then three successive matings with an alcoholic male failed to produce a viable offspring; one mating resulted negatively, one gave three young dying shortly after birth, and in the third case two late fetuses were aborted. She was then mated again to a normal male and produced two vigorous offspring.

Normal female No. 50 was mated alternately with normal and alcoholic males for 4 matings, with alternately good and bad results.

Animals 19, 30, 54 and 58, all normal females, show records closely similar to the ones just mentioned. (In a former table of successive matings the first mating of No. 19 has been recorded incorrectly; it should read by a normal male giving two normal offspring as in the present Table II, instead of by alcoholic male No. 4 with a negative result.)

Alcoholic female No. 64 gave two normal young by a normal male before her treatment was commenced. Mated to alcoholic male No. 6 she gave two young; one died at birth and one survived; with alcoholic male No. 5 she gave an apparently normal young; finally, by alco-

TABLE II

RESULTS OF THE SUCCESSIVE MATINGS OF FIFTEEN FEMALES

Animal	1st Mating	2d Mating	3d Mating	4th Mating	5th Mating	6th Mating	7th Mating	8th Mating
No. 64 alc.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 2 young, 1 died	Alc. ♂ 5, 1 nor. young	Alc. ♂ 43, 2 foetuses, died *in utero*, killed mother	.....	.....	.....	.....
No. 59 alc.	Nor. ♂, 3 nor. young	Nor. ♂ 44, 2 young, 1 died	Alc. ♂ 43, 0	Alc. ♂ 43, 0	Nor. ♂ 69, 3 yg., died *in ut.*, killed mother	.....	.....	.....
No. 30 nor.	Nor. ♂ 25, 2 nor. young	Alc. ♂ 45, 2 nor. young	Alc. ♂ 5, 1 nor. young	Alc. ♂ 5, 2 foetuses, died *in utero*, killed mother	.....	.....	.....	.....
No. 15 nor.	Alc. ♂ 6, 2 still-born	Alc. ♂ 5, 0	Alc. ♂ 5, 2 young, both died in convulsions	Nor. ♂, 1 nor. young	Alc. ♂ 5, 1 apparently normal	Alc. ♂ 43, 2 young, both died 2d day	Nor. ♂ 70, 2 nor. young	Ether ♂ 69, 2 young, 1 died
No. 19 nor.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 1 still-born	Alc. ♂ 6, 0	Alc. ♂ 5, 4 small, ½ size young	Alc. ♂ 45, 2 young, 1 died	Alc. ♂ 6, 0	Alc. ♂ 6, 3 young, 2 died	
No. 63 nor.	Nor. ♂ 44, 2 nor. young	Alc. ♂ 5, 0	Alc. ♂ 5, 3 young, all died	Alc. ♂ 5, 2 late foetuses aborted	Nor. ♂ 93, 2 nor. young	.....	.....	.....
No. 50 nor.	Nor. ♂ 46, 3 nor. young	Alc. ♂ 43, 2 young, both died	Nor. ♂ 69, 2 nor. young	Alc. ♀ 43, 0	.....	.....	.....	.....
No. 54 nor.	Nor. ♂ 44, 2 nor. young	Alc. ♂ 6, 2 young, both died	Nor. ♂ 72, 2 nor. young	Alc. ♂ 43, 1 young	.....	.....	.....	.....

Animal	1st Mating	2d Mating	3d Mating	4th Mating	5th Mating	6th Mating	7th Mating	8th Mating
No. 55 alc.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 0	Nor. ♂ 47, 2 young, both died	Alc. ♂ 6, 1 young	.....	.....	.....	.....
No. 56 nor.	Nor. ♂ 48, 2 nor. young	Alc. ♂ 45, 3 premature, stillborn	Nor. ♂ 80, 3 nor. young	Alc. ♂ 45, 1 young, very small	.....	.....	.....	.....
No. 58 nor.	Nor. ♂ 46, 2 nor. young	Alc. ♂ 6, 2 nor. young	Alc. ♂ 43, 1 young, died at birth	Alc. ♂ 6, 0	.....	.....	.....	.....
No. 60 alc.	Nor. ♂ 46, 3 young, all died	Nor. ♂ 44, 2 nor. young	Alc. ♂ 45, 1 stillborn	Alc. ♂ 43	.....	.....	.....	.....
No. 62 alc.	Nor. ♂ 47, 2 nor. young	Nor. ♂ 46, 2 small young	Alc. ♂ 6, 2 young, died 2d day	Alc. ♂ 45, 2 young, died 2d day	.....	.....	.....	.....
No. 65 nor.	Nor. ♂, 2 nor. young	Alc. ♂ 5, 2 small young	Alc. ♂ 6, 2 small young	Nor. ♂ 83, 2 young, 1 died, ♀ treated during pregnancy	.....	.....	.....	.....
No. 66 alc.	Nor. ♂, 1 nor. young	Nor. ♂ 44, 2 nor. young	Alc. ♂ 5, 0	2d gen. ♂ 102, 3 fetuses aborted	2d gen. ♂ 102, 2 small young	.....	.....	.....

holic male No. 43 she produced two fetuses which died *in utero* and killed her.

Female No. 59, alcoholic, has a similar record and was also finally killed by 3 fetuses dying *in utero* and poisoning her.

Alcoholic females 55, 60, 62 and 66 all show very poor records in the production of viable offspring.

During the course of the experiments four females have been killed by the death of fetuses *in utero*. In three of the cases this occurred after the females had been treated with alcohol for a number of months and were becoming more and more affected by the treatment. No. 61 died after she had been given alcohol for four months with three fetuses *in utero* which had apparently been dead for several days. No. 64 had been treated with alcohol for over one year and finally, while in a late stage of pregnancy by alcoholic male No. 43, she became stupid and refused to eat. On examination the fetuses showed no signs of life and were quite hard; they were removed by operation and had been dead several days. The mother had become so intoxicated by this condition that she was unable to recover after the removal of the fetuses.

Female No. 59 had been treated with alcohol for thirteen months when, after being mated with a normal male, she was operated upon in order to remove three dead fetuses. She failed to recover.

The fourth case of young dying *in utero* was that of a normal female, No. 30, that had been mated with an alcoholic male. The almost full term fetuses died and produced the same symptoms in the mother as those in the cases above; she was also operated upon and failed to recover.

It is a perfectly easy operation to remove the ovaries or uterus from a normal guinea-pig. I have not tried to remove living fetuses. There is little doubt, however, that it is the accumulated toxins owing to the presence of

the dead fetuses which prevented the females from recovering after the operation in these three cases.

The death of the late fetuses *in utero* is to be expected merely as a step in the series. A number of early abortions of embryos have occurred, and the table shows the enormous fatality among the young shortly after birth, as well as the frequent occurrence of stillborn litters. When the young happen to die shortly before birth instead of after birth, the female in some cases is unable to expel them from the uterus.

A consideration of a few of the notes made from the individual matings will further serve to illustrate the actual response of the animals to the treatment as shown by the outcome of the matings. For this purpose we may take two random groups.

First a group of eleven matings made on October 30 and 31, 1912, resulted as shown in the following notes:

Oct. 30, Nor. ♀ No. 30 × Alc. ♂ 5 = Jan. 9, 1913—One normal young, No. 123 ♀.

Oct. 30, Nor. ♀ No. 29 × Alc. ♂ 45 = Jan. 20, 1913—Three very small young, Nos. 134 ♂, 135 ♀, and 136 ♀.

Oct. 30, Nor. ♀ No. 58 × Alc. ♂ 6 = Jan. 15, 1913—Two normal young, Nos. 129 ♂, 130 ♀.

Oct. 30, Alc. ♀ No. 62 × Nor. ♂ 46 = Jan. 9, 1913—Two small but normal young, Nos. 121 ♀, 122 ♀.

Oct. 30, Alc. ♀ No. 59 × Alc. ♂ 43 = 0.—*Only one of the eleven matings that did not take.*

Oct. 30, Alc. ♀ No. 60 × Nor. ♂ 44 = Jan. 17, 1913—Two apparently normal young, Nos. 131 ♂, 132 ♂.

Oct. 31, Nor. ♀ No. 68 × Nor. ♂ 69 = Jan. 21, 1913—Three normal young, Nos. 137 ♂, 138 ♂, 139 ♂.

Oct. 31, Nor. ♀ No. 71 × Nor. ♂ 72 = Jan. 10, 1913—Two small normal young, Nos. 126 ♂, 127 ♂.

Oct. 31, Nor. ♀ No. 73 × Nor. ♂ 70 = Jan. 19, 1913—One large young, No. 133 ♀.

Oct. 31, Nor. ♀ No. 74 × Nor. ♂ 79 = Jan. 11, 1913—One large young, No. 128 ♀.

Oct. 31, Alc. ♀ No. 55 × Nor. ♂ 47 = Jan. 9, 1913—Two

small and weak young, Nos. 124 ♀, 125 ♂, both died when one month old.

In the group of 11 matings, 4 were between normal males and normal females, control matings, three were between alcoholic males and normal females, and three between normal males and alcoholic females. All of these produced offspring as noted, while the single mating between an alcoholic male and an alcoholic female gave a negative result although the animals were kept together as long as the individuals of any of the other pairs, 17 days.

One of the alcoholic females gave two young which died within a month.

A second group of 18 matings made during November, 1912, are recorded as follows:

Nov. 4, 2d Gen. ♀ 100 × 2d Gen. ♂ 92 = 0.—Together very long, though failed to take.

Nov. 4, 2d Gen. ♀ 101 × 2d Gen. ♂ 99 = 0.—Together very long, though failed to take.

Nov. 15, 2d Gen. ♀ 91 × 2d Gen. ♂ 92 = Feb. 18, 1913—One large young, apparently normal, No. 170 ♂.

Nov. 15, 2d Gen. ♀ 98 × 2d Gen. ♂ 99 = 0.—Failed to take, though together six weeks.

Nov. 16, 2d Gen. ♀ 76 × 2d Gen. ♂ 77 = Jan. 28, 1913—Three defective young, one eyeless, all died within 10 days.

Nov. 16, Nor. ♀ 88 × 2d Gen. ♂ 78 = Feb. 20, 1913—One large young apparently normal, No. 171 ♂ (long gestation).

Nov. 16, Alc. ♀ 66 × Alc. ♂ 5 = 0.

Nov. 16, Nor. ♀ 87 × 2d Gen. ♂ 75 = Jan. 27, 1913—Two normal young, Nos. 140 ♀, 141 ♂.

Nov. 16, Nor. ♀ 19 × Alc. ♂ 6 = Jan. 30, 1913—Three small weak young, two died shortly after birth, one No. 146 ♂ survived.

Nov. 16, Nor. ♀ 34 × Alc. ♂ 43 = Feb. 4, 1913—Two normal young, Nos. 156 ♀, 157 ♂.

Nov. 16, Nor. ♀ 49 × Alc. ♂ 45 = Jan. 31, 1913—Three normal young, Nos. 147 ♂, 148 ♀, 149 ♀.

Nov. 16, Nor. ♀ 33 × Nor. ♂ 46 = Jan. 30, 1913—Two fine young, Nos. 144 ♂, 145 ♀.

Nov. 23, Nor. ♀ 50 × Nor. ♂ 69 = Feb. 5, 1913—Two large young, Nos. 160 ♂, 161 ♀.

Nov. 23, Nor. ♀ 15 × Nor. ♂ 70 = Feb. 4, 1913—Two large young, Nos. 158 ♀, 159 ♂.

Nov. 23, Nor. ♀ 54 × Nor. ♂ 72 = Feb. 3, 1913—Two large young, Nos. 150 ♂, 151 ♂.

Nov. 23, Nor. ♀ 56 × Nor. ♂ 80 = Feb. 3, 1913—Three normal young, Nos. 152 ♂, 153 ♂, 154 ♀.

Nov. 23, Nor. ♀ 52 × Nor. ♂ 81 = Feb. 12, 1913—One large young, 167 ♀.

Nov. 23, Nor. ♀ 53 × Nor. ♂ 48 = Feb. 3, 1913—One large young, 155 ♂.

To summarize these 18 pairings: 7 were normal control matings all giving vigorous young. Three were alcoholic males by normal females, all gave young. Two litters consisted of small animals, while the third litter was very weak, two of its members died just after birth.

Five of the matings were made between untreated animals which came from alcoholic parents, second generation animals. One litter of 3 individuals was born, all were weak and defective, one being eyeless and the 3 died within ten days. One other litter consisted of only one individual which was born after an unusually long period of gestation. Three of the matings gave negative results. Thus 3 out of 5 second generation matings failed to take, one gave non-viable young, and only one litter of viable young was produced from the 5 matings, as against 7 viable litters from the 7 control matings.

There were two matings of second generation males by normal females. Both of these gave viable young, though one of the females had an unusually long gestation period. The normal mates seemed to have counteracted the weakened condition of the second generation males.

The one mating between two alcoholic individuals again gave negative results.

Thus 4 of the 18 matings failed to take, 3 of these were between second generation animals and the fourth was the double alcoholic mating.

Fourteen matings were successful, in five cases one member of the pair was normal and in seven cases both were normal. In the remaining two cases both animals were of the "second generation," though themselves untreated, one litter was non-viable and but a single litter of one young survived.

These sample notes from 29 pairs out of the total of 167 full term matings contained in Table I, gives a fairly clear idea of the manner in which the individual animals respond.

CONCLUSIONS

Finally, in conclusion, we may consider the type or nature of the injury produced by the treatments and the manner of transmission or inheritance involved. The treated animals themselves show no effects of nervous or systemic injuries in their general health or behavior. It is only when such individuals are bred that they prove to be inferior to the untreated animals. This inferiority is shown both by a slowness or failure in many cases to conceive, although they copulate normally, and by the poor quality of the offspring to which the successful conceptions give rise. That this poor quality of offspring is due to an injury inflicted by the treatment on the germ cells of the alcoholic animals is shown by the fact that when the male alone is treated the offspring he begets are decidedly inferior. The germinal taint is still further demonstrated by the fact that the offspring from treated parents although themselves not treated produce equally or more defective young than do the treated animals.

The defects shown by the offspring of alcoholic parentage are general in type, not definite or specific. The central nervous system and special sense organs are

apparently most affected, and this is true also in embryos developing in unfavorable environments. I have found that fish embryos when developed in a large number of unusual environments, including alcohol and ether, always show marked abnormalities of the nervous system and special sense organs, particularly of the eyes and ears. When chick embryos are subjected to similar environmental conditions, it has been found in experiments performed during the last two winters, that they respond in a manner similar to the fish. Many chick embryos show different degrees of cyclopia and the degeneration or absence of one eye of the normal pair is a common defect in the chick as it is in the fish where many grades of monophthalmicum asymmetricum were described in my communications on the subject. In this connection the eyeless guinea-pig derived from untreated animals that had an alcoholic father becomes of special interest, and the general nervous symptoms, spasms, epileptic-like seizures, etc., shown by animals of two generations gain importance.

All defects of the nature of those mentioned may be considered as due to weakened development or developmental arrest. Any environment which weakens or retards the early stages of development will cause such conditions. How, then, are they transmitted by the alcoholic male, or by the untreated offspring of alcoholic parentage?

When the animal is treated with alcohol, lead or almost any poison for a long period of time, the poison acts to weaken or injure all of the body tissues with which it comes in contact through the circulation, the liver and other glandular organs usually show the effects in particular. The reproductive glands are injured as well as others and all the cells and tissues of such an organ are below normal. When such a male animal is paired with a normal female, the resulting offspring contains in every cell of its body elements derived from the weak or injured male pronucleus. Unless the vigor of the normal parent

is sufficient to overcome the injured condition, the offspring is defective.

The important thing in considering this defective offspring is the recognition of the fact that not only its soma cells but its germ cells as well are defective, since all were derived from the modified spermatozoon of the injured father. When this offspring with injured germ cells is paired with a similar individual, as has been frequently done in the experiments described, the resulting animal body is constituted of cells, all of which are the result of proliferation or division from the primary injured egg and sperm cell; thus all of the cells are of a similar inferior nature. Therefore, the young derived from the second generation should be, leaving out of consideration the power of a cell to recover from such poisoning, equally as defective as those derived from the treated parents.

This might be construed to show the transmission of acquired characters, but it can not be properly interpreted in such a sense. There is in this case no transmission of new or strange characters strictly speaking, merely a weakened or injured cell gives rise to other weak cells. The term "weak" is employed for the lack of a better one, meaning that the cells are below normal in reaction, respond slowly or in a deranged manner and often die or wear out early in their career.

It may be that in nature such defects as hare-lip and cleft palate are transmitted in a fashion similar to the method just suggested. These defects run in families and are said to be inherited. Their character, however, is clearly that of a developmental arrest. Such defects are very probably not truly inherited at all, that is, they are not definite characters or qualities as hair and eye color are, but are due to the fact that the germ cells from which the deformed individual arose, or the uterine environment in which it developed, were not fully normal in vigor. A more careful study of the inheritance of such defects will doubtless reveal the fact that other deform-

ities and developmental arrests are also common in the same families. In other words, weak germ cells or the poor developmental environment runs in the family, and hare-lip and cleft palate are merely the external expressions of these conditions.

The interpretation may be concretely expressed as follows: Mammals treated with injurious substances, such as alcohol, ether, lead, etc., suffer from the treatments by having the tissues of their bodies injured. When the reproductive glands and germ cells become injured in this way they give rise to offspring showing weak and degenerate conditions of a general nature, and every cell of these offspring having been derived from the injured egg or sperm cell are necessarily similarly injured and can only give rise to other injured cells and thus the next generation of offspring are equally weak and injured, and so on. The only hope for such a line of individuals is that it can be crossed by normal stock, in which case the vigor of the normal germ cell in the combination may counteract, or at any rate reduce, the extent of injury in the body cells of the resulting animal. By continually introducing normal mates into such a line the defects might be entirely eliminated, but the continued interbreeding of animals with defects or systemic injuries will doubtless result in the death of the race.

The offspring from a diseased father derives all of its cells from the poor sperm, thus each cell is poor in part and is so passed from generation to generation.

The present experiments are being continued and a large number of matings between second and third generation animals are now made. Various combinations of second generation animals are being tried in order to compare the effects resulting from paternal and maternal treatments, as well as the doubled effects. Two animals, both derived from alcoholic fathers, are mated, others from alcoholic mothers, and the various crosses between these classes are tried. In other cases second generation sisters are mated one with a normal and the other with

an alcoholic male, and subsequently these matings will be reversed in order to study the power of the normal mate to counteract the injured condition, as well as the tendency of new alcoholic cells to augment the condition.

SUMMARY

Three years ago a series of experiments were begun with guinea-pigs in order to test the possibility of modifying the type of development in mammals, so as to produce definite monstrosities, as had been accomplished with lower vertebrates. This primary object has not been fully attained at the present time, yet the experiments have demonstrated several points concerning injury of the germ cells, and have shown that an alcoholized male guinea-pig almost invariably begets defective offspring even when mated with a vigorous normal female.

A method has been devised for administering the alcohol by inhalation. The animals inhale the fumes of 95 per cent. alcohol which are readily taken into the pulmonary circulation, and very soon cause a state of intoxication. By this method the stomach is not injured and the general metabolism of the animal is maintained in a healthy condition. Few changes are produced in the tissues of the animals, even after a treatment given six times per week has extended over almost three years. Yet the actual effects upon the reproductive glands are indicated by the inferior quality of the offspring to which the alcoholized individuals give rise.

The animals have been mated in various combinations. First, alcoholized males are paired with normal females, the paternal test, and also the crucial test of the influence of the treatment on the germ cells. Fifty-nine such matings have reached term. Twenty-five of these gave negative results or early abortions. Thirty-four of the fifty-nine matings resulted in conception which ran the full term. Eight, or about 24 per cent., of these were stillborn litters containing in all 15 dead individuals. Many of them were somewhat premature. Twenty six, or only 44

per cent., of the matings produced litters of living young, containing a total of 54. Twenty-one, or almost 40 per cent., of these young animals died within a few days or less than four weeks after birth and only 33 of them survived. Many of the 33 survivors are small excitable animals and though not treated themselves have usually given rise to defective offspring in the several cases where they have been mated with one another.

The second combination is between alcoholized females and normal males, the results of which are interesting in comparison with the above. In this combination there are two chances to injure the offspring; in the first place it may arise from a defective egg cell, or secondly, it may be injured by an abnormal developmental environment within the body of the alcoholized female. Fifteen such matings have been made. Three of these, or 20 per cent., gave negative results, or were possibly aborted very early. Three stillborn litters of nine individuals were produced. Sixty per cent. of the matings gave living litters, as against 44 per cent. in the first combination between treated males and normal females. The proportion of surviving young is, however, less from the treated females than from the treated males. Of 19 living young, 9 died soon after birth and 10 survived.

The third combination was between alcoholized males and females. Twenty-nine such matings gave in 15, or more than 50 per cent., of the cases negative results or early abortions. Three stillborn litters occurred, each consisting of two individuals. Only 11 living litters were produced containing 16 young, 9 of which survived while 7 died soon after birth.

All of the matings of the treated animals may be combined and compared with control matings as follows: In a total of 103 full term matings, 43, or almost 42 per cent., have given negative results or early abortions, while 35 control matings failed in only two cases, or about 6 per cent., to yield a full term litter. Fourteen, or 13½ per cent., of the matings gave stillborn litters consisting of

30 dead individuals. Only one stillborn litter occurred in the 35 control matings; this was a large litter of 4 individuals and the mother seemed almost unable to carry them. The 103 matings gave only 46 living litters, about 45 per cent., while 32 living litters, or 91½ per cent., were produced by the 35 control matings.

The 46 living litters from the alcoholic matings contained 89 young, 37 of which died shortly after birth and 52 survived. The 32 living litters from the normal animals consisted of 60 individuals only 4 of which died while 56, or 93 per cent., of them survived.

Of 119 full term young, living and stillborn litters, produced by the alcoholic animals only 52, or less than 44 per cent., survived as against the 56, or 87½ per cent., survivors among the 64 full term control offspring.

The offspring derived from the alcoholic individuals are termed second generation animals and were not themselves treated with alcohol. In three cases second generation individuals have been mated with normal and have given perfect results, although the litters have been small. It might seem as though the normal mate possessed a strong tendency to counteract any defect which may have been present in the second generation animal.

Mating second generation individuals with alcoholized guinea-pigs gave very different results. Two out of three such matings produced stillborn young, one of which was grossly deformed. The third mating gave two surviving young.

Nineteen matings have been made between second generation animals, the outcome of which compares very unfavorably with that from the control matings, while the data are closely similar to those obtained from the alcoholic matings. Seven, or almost 37 per cent., of the matings gave negative results. Twelve living litters were born consisting of 19 individuals, 6, or about 32 per cent., of which died very soon after birth and showed various nervous disorders; one was entirely eyeless and decidedly deformed.

From the number of records available one might conclude that the effects of the alcoholic treatment were as pronounced upon the offspring of the second generation animals, although they had not been directly treated, as upon the offspring of alcoholized individuals. The poison injures the cells and tissues of the body, the germ cells as well as other cells, and the offspring derived from the weakened or affected germ cells have all of the cells of their bodies defective, both soma and germ, since each of the cells is a descendant of the injured germ cell combination. In this manner the defects or degenerate conditions are transmitted or passed to subsequent generations.

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SUPPLEMENTARY STUDIES ON THE DIFFERENTIAL MORTALITY WITH RESPECT TO SEED WEIGHT IN THE GERMINATION OF GARDEN BEANS

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I. INTRODUCTORY REMARKS

IN an earlier paper[1] I have shown that in field cultures the mortality of seeds of *Phaseolus vulgaris* is not random, but selective with respect to seed weight.

From the available data it appeared that both the upper and lower weight classes are more heavily drawn upon in the mortality than is the modal region of the seed weight distribution. So delicately balanced is this mortality of the two extremes in the particular series of experiments that the mean weight of the survivors in the long run differs not at all from that of the population from which they are drawn, while their variability, either absolute as measured by the standard deviation or relative as expressed by the coefficient of variation, is distinctly less than that of the original population.

Now while these results are deduced from such large and representative series of data, that their general validity for the specified conditions seems beyond much question, their substantiation has appeared to me for two reasons most desirable:

(*a*) The demonstration of selective elimination was somewhat indirect. Comparisons could not be made between the physical constants of the seeds which developed and those of all exposed to risk, or between the constants of those which actually failed and those which actually developed, but were necessarily drawn between the constants of the general population from which the seeds were taken for planting and those of the seeds

[1] Harris, J. Arthur, "On Differential Mortality with Respect to Seed Weight Occurring in Field Cultures of *Phaseolus vulgaris*," AMER. NAT., 46: 512–525, 1912.

which developed to maturity. The method is perfectly legitimate, providing the samples planted be drawn in a purely random manner (as they were in these experiments), but the probable error of random sampling is a two-fold one, and this increases the difficulty of determining the statistical significance of a given constant.

(*b*) The result seemed, *a priori*, improbable. Other studies[2] had demonstrated a moderately low but consistently positive correlation between the weight of the seed planted and the number of pods on the plant produced. It seemed reasonable to assume that since the larger seed produce the heaviest plants they are in general more vigorous, and hence should be more viable.[3] If the seeds increase in vigor and viability from the smallest to the largest, one would anticipate an increase in the mean of the survivors and a decrease in their variability resulting from the mortality in the lower part of the range of variation instead of a reduction in variability without a change in type (mean). These were the biological hypotheses which led me to question the generality of the statistical findings. Further work was therefore undertaken along various lines. Additional field cultures in which it will be possible to compare the constants of the seeds developing with those of the seeds failing, were made.[4] Such cultures can only substantiate, refute or modify the conclusions drawn from the experiments already carried out, but will not advance our knowledge of the proximate causes of the differential mortality. To this end, physiological (including chemical and physical) studies must be instituted.

The purpose of this paper is to discuss the results of

[2] Harris, J. Arthur, "On the Relationship between the Weight of the Seed Planted and the Characters of the Plant Produced," *Biometrika*, 9, pt. 1, 1913; also "The Size of the Seed Planted and the Fertility of the Plant Produced," *Amer. Breed. Mag.*, 3: 293–295, 1912.

[3] Providing of course that the correlation between size of seed planted and size of plant produced is not merely the result of extra reserve food in the larger seeds.

[4] The results of these and of other data from experiments made long since, but as yet in a raw condition, should be ready in a few months.

one of these physiological experiments, in as far as they bear upon the questions of the existence of a differential mortality and of its consequences in the population. The evidence which they afford concerning the causes underlying the differential death rate is a question too complicated both biologically and statistically to be discussed in the limits of this paper.

For a fair understanding of the portions of the data which are placed before the reader, it will be necessary, however, to state briefly the general purposes which led to the adoption of the particular methods employed.

On the assumptions that the vigor of the seeds increases from the lower to the higher weight classes,[5] one might expect a mortality of seeds in the lower portion of the range of variation due to innate incapacity for development. One must then seek some other factor to account for the mortality of the heavier seeds.

One of the simplest *a priori* assumptions is that the larger seeds require longer to germinate and that they are in consequence longer exposed to the vicissitudes of germination—to death by excessive moisture or by excessive draught before or shortly after expanding their leaves.

Now nothing whatever is here stated or implied in favor of any of these suggestions. For the present, they stand purely and simply as the first of a series of hypotheses to be tested in the quest of the true interpretation of an observed phenomenon. They are mentioned here solely to explain why a particular series of experiments was set up in the way in which it was.

II. Methods

The first thing needful in testing these hypotheses is to determine the relationship between the size of the seed and the time required for its germination. To do this, while at the same time securing data for a further test of

[5] The chief evidence in support of this view is that afforded by the results already mentioned for the correlation between the weight of the seed planted and the characters of the plant produced. But of course this correlation may be due solely to stored food materials.

the existence of a selective mortality, one must work with as large numbers of seeds as possible in order to obtain a reliable measure of selective mortality as well as decisive constants for the relationship between seed weight and time required for germination, if it be of the low or moderate order that one might expect. It is desirable that the germination tests be made under conditions as uniform as possible. The technique adopted must also be practical—that is, in the case of the present study, the work was necessarily done at a season of the year when it would not interfere with other experiments; the seeds had to be germinated so that each of the many hundreds or thousands of pots could be examined without too great back or eye strain every three hours throughout the twenty-four during the whole period of germination; finally, the expense of setting up and maintaining the experiment had to be kept within reasonable limits.

These requirements seemed, after careful consideration, best met by planting the seeds separately in three-inch pots of moderately fine sand. To facilitate handling, the pots were filled with slightly moist sand which was generally allowed to dry before the seeds were planted. The whole experiment was then watered at the same time. In a few instances, it was impossible to have the sand of all the pots perfectly dry when the seeds were planted, but I believe this introduces only a small source of error, for in these cases the planting was rushed through as rapidly as possible, and the individually labeled seeds were always thoroughly shuffled before planting to counteract, in as far as might be, the heterogeneity of environmental conditions afforded by different parts of the greenhouse. The space on the benches was filled to the level of the top of the pots with sand to prevent too great evaporation.[6] The labels were com-

[6] In a few earlier experiments, fine bench gravel was used, in the later ones, sand of the same kind as that in the pots. The gravel was employed at first, since I thought it might be feasible to water indirectly by flooding the gravel and allowing the sand to absorb it through the sides of the pots. This proved entirely impracticable. Not only was the method of watering unsuccessful, but the gravel permitted an enormous amount of evaporation.

pletely sunk in the sand so that the series number and the weight of the seed were quite unknown at the time of recording the results. Thus personal equation as far as it implies any bias with regard to the material was absolutely excluded.

At the outset, I must emphasize the fact that this technique (which I still believe is the best possible under all the requirements) falls far short of what one would desire. The germination of bean seeds under glass on a large scale is a rather difficult process. If a sufficient supply of moisture can be held in the soil from the beginning to the end of the experiment and the temperature be kept fairly high, the problem of good germinations in the greenhouse is solved. But when one is doing the work during the period of hot days and cool nights coming in the early fall, the question of maintaining proper soil moisture and temperature is a very serious one. It is remarkable how heterogeneous the environment of a single section of a greenhouse system is! This is especially noticeable in the drying out of the pots in sand cultures. Just here lies one of the greatest difficulties. The germinating bean seedling is very sensitive to watering, especially in connection with low temperature. I imagine this is particularly true of old seeds which have nearly lost their viability. Probably the considerable irregularity in our percentages of germination is very largely due to the impossibility of controlling closely enough the soil moisture.[7]

In classifying, three groups were recognized: (*A*) seeds germinating normally, (*B*) seeds germinating but producing seedlings more or less abnormal, (*C*) seeds failing to germinate.

On general grounds, the recognition of the three classes seemed desirable; for purposes other than those of this paper, it was essential. They can, of course, be combined

[7] The effect of this inability to control moisture sufficiently was, when present, always in the direction of a reduction of the percentage of germination through the rotting of some of the seeds, for in all cases the amount of water was finally sufficient to bring about germination.

at pleasure for comparisons. The distinction between *A* and *C* or *B* and *C* allows of practically no difference of opinion. Personal equation probably plays considerable part in distributing the seedlings between those which germinated normally and those which were somewhat abnormal, for there is no clear line of distinction between the two. Practically all the cases were decided by myself. The abnormalities were in small part teratological and in part physiological or pathological—*i. e.*, curved hypocotyls failing to bring the plumule promptly to the surface, cotyledons failing to free themselves from the seed coat, blighted primordial leaves, etc. The results of this study seem to indicate the need of more precise consideration of aberrant seedling in future experiments.

III. MATERIALS

This research and the one which preceded it are in-between seed weight and seed mortality in *Phaseolus vulgaris* as a whole,[8] and at the same time lay up data which when sufficiently supplemented by others of various kinds shall enable one to determine whether (and if so, why) the relationship between seed weight and seed mortality differs from variety to variety, or whether it is dependent upon the conditions under which the seeds planted were grown or those under which they were germinated, or upon the age of the seeds.

Five characteristics were, therefore, deemed desirable in the seeds used. (*a*) They should be known from breeding tests to belong to strains as uniform as possible. (*b*) They should represent several distinct varieties. (*c*) Different lots should have been grown under as diverse environmental conditions as possible. (*d*) Different ages of as nearly as possible comparable seed should be investigated. (*e*) Comparison with the results of field experiments should be easily carried out.

[8] The materials are, however, for technical reasons limited to the dwarf varieties.

These conditions were most satisfactorily met in the seeds held over from various pedigree experiments made during the last several years. Coupled with the favorable points of these are some obvious disadvantages,[9] which practically are of relatively small weight in view of the fact that it would require several years work to secure a better series.

It is unnecessary to devote space to the description of these materials, since the key letters used are those employed in previous papers, in which a large amount of quantitative information concerning them may be obtained.

Altogether thirteen "experiments" were made. That is, a greenhouse or a section of a greenhouse was filled thirteen times. These experiments are numbered *A* to *M*, and the letters separated from the pedigree formulæ by dashes in the tables refer to them. As a glance at the tables will show, several different series of seeds often went into a single experiment—the capacity of the small greenhouse being about 3,000 and that of the large greenhouse about 8,000 pots. The specific details of these experiments seem at present irrelevant.

[9] Chief among these is the age of some of the seeds—resulting in very low percentages of germination. This is possibly a very important factor. The field cultures were grown in 1908, 1909 and 1910. The sand cultures, made in large part from samples of the same lots of seeds as used in the various field experiments, were carried out in the summer of 1912. Any one who takes the ratio of the seeds germinating to those actually planted for the individual samples will be impressed by the very low percentages of germination in these experiments. This is largely attributable to differences in age of seed, but in addition it will be noted that the seeds were grown under different environmental conditions and that they were germinated under conditions which could not be maintained the same from experiment to experiment. Inability to control temperature and substratum moisture may account for considerable differences.

Now it is clear that in these experiments it has not been possible to differentiate between the deaths which occurred in the seed envelopes and those which have taken place under the vicissitudes of field or sand culture conditions. This problem can not be profitably discussed until experiments under varying and carefully controlled conditions can be made with seed identical except for age. For such experiments one should start with large quantities of pedigreed seed and follow it through its period of viability. Material was bred for this purpose in the summer of 1912.

IV. ANALYSIS OF DATA

The distributions of seed weight are shown in the conventional units of .025 gram range.[10] Tables I–II give those for the seeds germinating normally, Tables III–IV for those which germinated but produced more or less abnormal seedlings,[11] Tables V–VI those for seeds which failed to germinate.

From these the three more essential physical constants (mean, standard deviation and coefficient of variation) have been deduced and are presented with their probable errors in Tables VII, VIII and IX.[12]

TABLE I

WEIGHT OF SEEDS GERMINATING NORMALLY

Series	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Totals
NHD–J.....	—	2	4	8	14	18	12	9	3	6	—	1	—	—	77
NHD–M.....	—	2	3	14	23	58	37	25	5	2	1	—	—	—	170
NHH–J.....	1	—	—	7	29	38	71	27	16	13	6	—	—	1	209
NHH–M.....	—	—	5	30	88	158	186	120	50	19	11	6	3	—	676
NHHH–J.....	—	7	33	120	215	252	122	45	16	5	—	—	1	1	817
NHHH–M....	2	12	47	176	389	456	218	88	25	5	1	—	—	—	1,419
NHDD–J.....	—	1	8	42	140	233	225	142	51	17	8	—	—	—	867
NHDD–M....	1	5	20	59	202	344	318	175	58	16	5	6	—	—	1,209
NDH–D.....	—	—	4	7	48	56	43	16	2	—	—	—	—	—	176
NDH–E.....	—	1	1	31	65	87	54	16	3	—	—	—	—	—	258
NDD–D.....	—	3	2	10	21	19	16	13	3	1	—	—	—	—	88
NDD–E.....	—	—	4	5	16	17	12	3	—	—	—	—	—	—	57
NDDD–D.....	—	1	8	62	108	122	60	22	4	—	—	—	—	—	387
NDDD–E.....	—	—	6	67	106	98	53	35	7	—	2	1	—	—	375
NDHH–D....	1	7	17	66	144	153	98	25	2	1	—	—	—	—	514
NDHH–E.....	—	4	10	50	122	154	81	24	6	—	—	—	—	—	451
FSS–I.....	5	56	202	268	121	34	14	—	—	—	—	—	—	—	700
FSS–L.....	2	63	328	467	252	59	9	—	—	—	—	—	—	—	1,180
FSH–C.....	—	—	8	51	183	164	52	11	3	—	—	—	—	—	472
FSD–C.....	—	7	43	104	52	19	7	—	—	—	—	—	—	—	232
FSHH–C.....	4	34	178	317	223	89	20	2	—	—	—	—	—	—	867
FSDD–C.....	1	27	107	259	175	89	20	2	—	—	—	—	—	—	680

[10] Class 1 = 0.000–0.025 gram, class 2 = 0.025–0.050 gram, etc. Thus to pass from the constants (means or standard deviations) in units to those in grams subtract .5 and multiply by .025.

[11] In some of these series, N is insignificant, but it has seemed best to lay the whole data before the reader. The degree of trustworthiness of the constants is indicated by their probable errors. In some cases, too, lots of material are combined.

[12] Tables of constants for $(A + B)$ and $(B + C)$ are not given, although they enter into some of the comparisons. They can be derived from the original tables of data or calculated from the constants for A, B and C by appropriate formulæ.

TABLE II

Weight of Seeds Germinating Normally

Series	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Totals
USS–I	—	1	1	1	13	39	106	142	162	176	135	120	78	47	18	21	9	4	3	3	3	1	1	—	—	1	—	—	—	—	—	1,085
USS–K	—	—	—	2	5	16	41	90	141	167	206	181	107	88	37	10	3	3	—	—	—	—	—	1	—	—	—	—	—	—	—	1,098
GGH–F	—	1	—	—	—	—	—	2	6	17	27	51	81	94	86	78	57	39	27	18	7	2	1	—	—	—	—	—	—	—	—	594
GGH–G	—	—	—	—	—	—	—	—	—	3	10	16	33	32	32	23	17	14	4	2	3	—	1	—	—	1	—	—	—	—	—	191
GGH–K	—	—	—	—	—	1	—	2	6	13	20	41	57	60	60	61	54	29	14	10	4	1	1	1	—	—	—	—	—	—	—	435
GGH$_2$*–F*	—	—	—	—	—	1	1	3	1	8	22	29	76	77	58	55	45	26	10	8	5	—	—	—	—	—	—	—	—	—	—	425
GGH$_2$*–G*	—	—	—	—	—	—	2	5	2	4	19	20	41	38	41	23	16	19	7	7	3	1	—	—	—	—	—	—	—	—	—	248
GGH$_2$*–K*	—	—	—	—	—	—	—	2	7	6	12	27	30	51	50	44	54	29	15	6	4	1	3	—	—	—	—	—	—	—	—	341
GGD–F	—	—	—	—	1	4	5	12	34	34	41	39	20	13	9	6	2	—	—	1	1	—	—	—	—	—	—	—	—	—	—	222
GGD–G	—	—	—	—	2	3	2	13	11	27	17	22	5	5	9	2	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	121
GGD–K	—	—	—	1	1	4	9	18	47	41	32	31	21	13	8	6	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	237
GGD$_2$*–F*	—	—	—	1	1	1	2	12	19	42	70	77	64	44	29	16	12	8	3	—	—	—	—	—	—	—	—	—	—	—	—	401
GGD$_2$*–G*	—	—	—	—	—	1	2	11	22	23	58	43	61	33	25	9	11	1	2	—	—	—	—	—	—	—	—	—	—	—	—	302
GGD$_2$*–K*	—	—	—	—	2	2	2	11	25	34	47	68	54	55	54	25	21	5	6	1	1	—	—	—	—	—	—	—	—	—	—	413
GGHH–F	—	—	—	—	—	—	—	1	5	8	14	38	48	56	41	28	18	22	20	2	—	1	1	—	—	—	—	—	—	—	—	303
GGHH–G	—	—	—	—	—	—	—	1	1	5	7	8	18	28	15	12	9	6	4	1	1	—	—	—	—	—	—	—	—	—	—	116
GGHH–K	—	—	—	—	—	—	—	1	5	9	16	28	54	62	38	29	16	15	12	3	2	2	—	—	—	—	—	—	—	—	—	292
GGDD–F	—	—	—	—	4	1	2	8	16	28	61	37	59	51	35	8	9	1	2	—	—	—	—	—	—	—	—	—	—	—	—	322
GGDD–G	—	—	—	—	1	—	1	1	6	16	22	22	22	17	9	7	5	2	3	—	—	—	1	—	—	—	—	—	—	—	—	135
GGDD–K	—	—	—	—	1	2	3	7	22	36	65	63	52	30	22	16	5	3	1	1	—	—	—	—	—	—	—	—	—	—	—	329
LL–A	—	—	—	—	6	6	5	3	8	10	18	8	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	69
LL–B	—	—	—	—	—	3	1	4	9	5	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	26
LLS–A	—	—	4	11	13	25	33	51	37	48	37	43	24	13	9	10	4	1	—	1	1	—	—	—	—	—	—	—	—	—	—	365
LLS–B	—	—	3	8	9	13	24	29	17	30	24	16	12	8	3	2	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	200
LLS–H	1	3	10	34	75	165	207	272	299	303	259	204	153	87	51	40	26	13	2	3	—	—	—	—	—	—	—	—	—	—	—	2,207
LLS–K	—	—	1	6	8	10	22	35	38	43	26	22	12	8	6	3	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	243
W–K	—	—	—	—	—	1	2	7	14	20	39	42	57	60	56	61	55	34	33	19	13	10	11	6	3	2	2	1	—	—	1	549
GGS–L	—	—	—	—	—	—	—	—	1	3	5	10	20	11	29	27	22	19	11	10	4	6	1	—	—	—	—	—	—	—	—	179

TABLE III

WEIGHT OF SEEDS MORE OR LESS ABNORMAL IN GERMINATION

Series	4	5	6	7	8	9	10	11	12	13	14	15	Totals
NHD–J	—	1	4	6	9	14	12	4	—	3	—	—	53
NHD–M	—	1	—	5	18	24	26	11	3	1	—	—	89
NHH–J	—	—	—	1	13	13	16	11	4	—	—	—	58
NHH–M	—	—	—	1	17	42	39	26	14	6	1	2	148
NHHH–J	1	—	2	7	7	15	6	2	1	—	—	—	41
NHHH–M	1	1	2	7	10	17	8	7	—	—	—	—	53
NHDD–J	—	1	2	6	14	9	12	5	5	—	—	—	54
NHDD–M	—	—	—	4	12	24	10	3	2	2	—	—	57
NDH–D	—	—	—	10	32	28	30	11	—	—	1	—	112
NDH–E	—	—	1	17	27	40	26	10	4	1	—	—	126
NDD–D	2	1	2	4	9	19	8	6	—	—	—	—	51
NDD–E	—	—	1	3	10	9	10	2	3	—	—	—	38
NDDD–D	—	—	1	11	15	15	11	3	1	—	—	—	57
NDDD–E	—	—	2	8	9	4	5	1	—	1	—	—	30
NDHH–D	1	—	3	6	22	14	11	4	1	—	—	—	62
NDHH–E	—	—	1	6	6	14	12	1	1	—	—	—	41
FSS–I	1	7	24	28	6	1	—	—	—	—	—	—	67
FSS–L	—	8	30	33	24	11	4	—	—	—	—	—	110
FSH–C	—	—	—	12	39	39	24	10	2	—	—	—	126
FSD–C	—	5	10	28	12	1	—	—	—	—	—	—	56
FSHH–C	—	5	8	21	12	6	—	—	—	—	—	—	52
FSDD–C	1	2	7	20	13	3	1	—	—	—	—	—	47

Any conclusion concerning selective mortality must rest upon a comparison of these constants.

The method of making these tests demands a word of explanation. In the previous study, the comparison was necessarily drawn between the constants of the seeds which actually produced fertile plants and those of the general population from which they were drawn; the constant for the general population was subtracted from that of the sub-sample. The positive or the negative sign of the difference showed whether mortality had tended to raise or to lower mean or variability.

In these greenhouse experiments, on the other hand, we have the constants for samples (*A*) normally germinating, (*B*) germinating abnormally and (*C*) failing to germinate. (*B*) may possibly be regarded as intermediate between (*A*) and (*C*).

If we take the difference between the constants

Survivors *less* failed

we shall have plus differences of the mean if selection has tended to raise the general average by eliminating the

TABLE IV

WEIGHT OF SEEDS MORE OR LESS ABNORMAL IN GERMINATION

Series	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Totals
USS–I	—	—	—	—	7	4	7	9	9	4	2	5	2	—	2	—	—	—	1	1	—	—	—	—	—	—	—	53
USS–K	—	1	1	—	3	2	10	8	15	16	17	7	10	4	—	—	—	—	—	—	—	—	—	—	—	—	—	94
GGH–F	—	—	—	—	—	—	1	2	1	2	7	14	26	30	16	19	13	8	2	2	—	—	—	—	—	—	—	143
GGH–G	—	—	—	—	—	—	1	1	—	3	4	4	11	13	6	9	5	1	3	1	1	—	—	—	—	—	—	63
GGH–K	—	—	—	—	—	—	2	1	1	2	5	9	14	15	6	14	10	4	3	2	1	—	—	—	—	—	—	89
GGH$_2$–F	—	—	—	—	—	—	—	—	—	—	1	2	3	4	3	4	1	5	1	1	—	—	—	—	—	—	—	25
GGH$_2$–G	—	—	—	—	—	—	—	—	—	—	3	2	3	3	6	2	1	—	2	1	1	—	—	—	—	—	—	24
GGH$_2$–K	—	—	—	—	—	—	—	1	—	1	—	—	1	—	1	—	2	—	—	—	—	—	—	—	—	—	—	6
GGD–F	—	—	—	—	1	9	13	16	30	29	9	5	7	1	1	—	1	—	—	—	—	—	—	—	—	—	—	122
GGD–G	—	1	—	—	1	1	9	15	13	23	15	8	9	2	2	1	—	—	—	—	—	—	—	—	—	—	—	100
GGD–K	—	—	—	—	3	4	23	41	54	44	27	15	9	4	4	—	—	—	—	—	—	—	—	—	—	—	—	228
GGD$_2$–F	—	—	—	—	1	1	2	3	2	3	6	6	3	—	1	—	—	—	—	—	—	—	—	—	—	—	—	28
GGD$_2$–G	—	—	1	1	—	—	—	3	2	3	1	6	4	3	1	2	—	1	—	—	—	—	—	—	—	—	—	28
GGD$_2$–K	—	—	—	—	—	—	—	2	2	1	4	5	2	1	2	—	1	—	—	—	—	—	—	—	—	—	—	20
GGHH–F	—	—	—	—	—	—	—	1	1	—	1	2	1	7	1	1	1	3	—	—	—	—	—	—	—	—	—	19
GGHH–G	—	—	—	—	—	—	—	—	—	—	2	1	3	1	1	1	2	—	—	—	1	—	—	—	—	—	—	12
GGHH–K	—	—	—	—	—	—	—	—	2	1	1	—	—	1	1	1	1	—	—	—	1	—	—	—	—	—	—	9
GGDD–F	—	—	—	—	—	—	1	2	1	—	—	1	4	1	1	—	—	—	—	—	—	—	—	—	—	—	—	11
GGDD–G	—	—	—	—	—	—	—	—	1	—	2	2	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	8
GGDD–K	—	—	—	—	2	—	1	—	1	—	2	3	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	12
LL–A	—	—	—	—	1	2	4	8	11	8	14	2	6	3	—	—	—	—	—	—	—	—	—	—	—	—	—	59
LL–B	—	—	—	—	1	2	2	10	4	4	6	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33
LLS–A	—	1	—	1	3	4	4	8	10	14	6	15	13	19	13	5	7	4	2	2	—	—	—	—	—	—	—	131
LLS–B	—	—	—	2	—	2	1	6	4	9	6	2	4	9	4	2	2	2	—	—	—	—	—	—	—	·	—	55
LLS–H	·	2	7	13	12	34	44	47	73	77	80	119	109	101	49	40	34	20	12	2	6	1	—	—	—	—	—	886
LLS–K	—	1	1	3	3	5	10	6	16	10	16	20	18	13	12	7	3	3	—	—	—	—	—	—	—	—	—	147
W–K	—	—	—	—	—	—	1	1	3	3	6	3	1	3	9	6	3	4	4	7	2	—	1	—	2	—	1	60
GGS–L	—	—	—	—	—	1	—	2	4	1	16	21	22	42	38	36	34	17	10	2	2	2	—	1	—	—	—	251

TABLE V

WEIGHT OF SEEDS FAILING TO GERMINATE

Series	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Totals
NHD–J.....	—	15	75	180	370	338	205	90	22	6	1	1	—	—	—	1,303
NHD–M.....	—	10	82	213	397	442	277	92	33	5	—	—	—	—	—	1,551
NHH–J.....	—	—	7	26	157	329	359	222	97	31	11	4	—	—	1	1,244
NHH–M.....	—	3	6	36	157	384	463	289	108	33	12	5	3	1	—	1,500
NHHH–J....	2	7	23	82	164	201	95	31	15	3	1	—	—	—	—	624
NHHH–M...	1	6	16	30	50	62	35	14	3	1	—	—	—	—	—	218
NHDD–J....	—	2	10	33	92	116	112	69	24	7	2	—	—	—	—	467
NHDD–M....	—	1	9	21	55	65	32	17	4	2	1	—	—	—	—	207
NDH–D.....	1	1	17	67	141	145	102	1	13	2	—	—	—	—	—	490
NDH–E.....	—	3	16	75	196	214	123	55	11	1	—	—	—	—	—	694
NDD–D.....	2	6	24	95	162	137	75	20	10	—	—	—	—	—	—	531
NDD–E.....	1	3	32	84	147	137	75	27	5	—	—	—	—	—	—	511
NDDD–D....	—	2	8	33	64	65	51	15	5	1	—	—	—	—	—	244
NDDD–E....	—	3	8	35	73	60	31	16	—	1	1	—	—	—	—	228
NDHH–D....	—	1	13	53	101	91	52	23	2	—	—	—	—	—	—	337
NDHH–E....	—	1	10	23	73	74	39	21	—	—	—	—	—	—	—	241
FSS–I.....	13	164	254	264	92	21	3	2	—	—	—	—	—	—	—	813
FSS–L.....	13	104	298	243	143	50	11	1	—	—	—	—	—	—	—	863
FSH–C.....	—	1	6	57	139	144	52	13	4	1	—	—	—	—	—	417
FSD–C.....	4	44	164	197	91	27	3	—	1	—	—	—	—	—	—	531
FSHH–C.....	1	12	62	114	93	31	10	3	—	—	—	—	—	—	—	326
FSDD–C.....	—	24	92	113	72	20	7	2	1	—	—	—	—	—	—	331

smaller seeds; we shall have negative differences for standard deviations and coefficients of variation if there is a mortality of both the larger and smaller seeds—thus increasing the variability in the eliminated sample and decreasing variability in the surviving population.

Hence, regarding the abnormal germinations (tentatively) as intermediate between normal development and failure, we take our differences:

(A)–(C), or normally germinating *less* failed,

(B)–(C), or abnormal *less* failed,

(A)–(B), or normally germinating *less* abnormal.

Since the number of individual experiments is fairly large, the comparisons may be made by merely noting the sign of the differences—*i. e.*, by taking the gross results of the individual experiments. Or one may treat the data from a more numerical view-point, taking averages of the actual differences. Both methods will of course be used.

In considering the differences between the constants of the three classes of seeds dealt with for the whole experi-

TABLE VI

Weight of Seeds Failing to Germinate

Series	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Totals
USS–I	—	—	2	3	6	18	25	46	73	69	53	54	51	16	15	9	4	3	—	1	—	—	—	—	—	—	448
USS–K	—	—	—	2	3	3	9	27	20	40	47	50	30	20	9	6	3	1	1	—	—	—	—	—	—	—	271
GGH–F	—	—	—	—	—	—	1	4	2	4	10	17	15	31	34	31	29	20	29	8	5	—	2	—	—	1	243
GGH–G	—	—	—	—	—	—	—	4	3	9	11	18	25	44	40	42	29	25	9	7	3	3	4	—	2	—	278
GGH–K	—	—	—	—	—	—	—	2	2	3	7	13	16	30	31	36	25	12	5	7	4	1	1	1	1	—	197
GGH$_5$–F	—	—	—	—	—	1	—	—	1	—	4	7	12	6	8	6	8	5	1	2	1	—	—	—	—	—	62
GGH$_5$–G	—	—	—	—	—	—	1	3	—	—	4	9	3	13	21	12	10	5	2	—	2	—	—	—	—	—	85
GGH$_5$–K	—	—	—	—	—	—	—	1	—	2	—	5	2	10	13	8	8	4	2	1	1	—	—	—	1	—	58
GGD–F	—	—	—	2	2	—	5	4	16	16	13	16	7	1	—	1	—	—	1	—	—	—	—	—	—	—	84
GGD–G	—	—	—	2	2	6	13	21	24	28	27	21	20	14	5	2	1	2	—	—	—	—	—	—	—	—	188
GGD–K	—	—	—	1	—	5	7	12	26	38	37	23	22	7	6	2	1	—	—	—	—	—	—	—	—	—	187
GGE$_5$–F	—	—	—	1	—	—	3	1	5	4	9	7	14	3	3	—	—	—	—	—	—	—	—	—	—	—	50
GGD$_5$–G	1	—	1	2	5	1	2	9	6	7	5	18	25	15	9	1	4	4	—	—	—	1	—	—	—	—	116
GGD$_5$–K	—	—	—	—	—	—	2	5	2	7	6	13	7	8	6	3	3	—	1	—	—	—	—	—	—	—	63
GGHH–F	—	—	—	—	—	—	—	1	—	1	1	10	9	8	15	3	3	—	1	3	1	—	—	—	—	—	56
GGHH–G	—	—	—	—	—	—	—	—	—	—	5	3	8	5	6	7	2	3	2	3	1	—	—	—	—	—	45
GGHH–K	—	—	—	—	—	—	—	—	—	—	3	5	2	10	2	4	7	1	4	1	1	1	—	—	—	—	41
GGDD–F	—	—	—	—	—	—	2	2	2	3	3	10	—	4	1	1	—	—	—	—	—	—	—	—	—	—	29
GGDD–G	—	—	—	—	—	—	1	1	2	8	9	8	12	2	3	3	1	1	—	—	—	—	—	—	—	—	52
GGDD–K	—	—	—	—	—	—	—	2	5	9	8	5	2	6	3	1	—	1	1	1	—	—	—	—	—	—	45
LL–A	—	—	2	6	9	14	[illegible]	29	51	66	65	42	30	24	18	5	4	1	—	—	—	—	—	—	—	—	396
LL–B	—	—	—	—	7	16	41	[illegible]	68	85	86	57	42	16	9	—	1	3	1	—	—	—	—	—	—	—	487
LLS–A	—	—	3	2	4	9	9	9	[illegible]	10	8	9	9	7	8	5	1	1	—	—	1	1	1	—	—	—	105
LLS–B	—	—	3	9	9	16	28	24	36	[illegible]	37	23	24	25	23	16	11	10	4	2	—	1	—	—	—	—	327
LLS–H	—	—	3	15	18	24	15	34	34	46	[illegible]	43	49	56	61	44	38	21	13	12	5	4	—	3	—	—	581
LLS–K	—	—	—	3	1	3	3	4	3	3	2	[illegible]	3	7	7	9	2	3	1	—	1	—	—	—	—	—	58
W–K	—	—	—	—	—	1	1	3	5	6	9	3	[illegible]	5	14	7	9	8	5	8	4	—	2	—	2	—	98
GGS–L	—	—	—	—	—	—	1	1	6	6	5	28	41	[illegible]	86	92	86	66	53	25	15	19	9	2	3	1	609

TABLE VII

PHYSICAL CONSTANTS FOR SEEDS GERMINATING NORMALLY

Series	Mean and Probable Error	Standard Deviation and Probable Error	Coefficient of Variation and Probable Error
NHD–J	9.247 ± .154	2.006 ± .109	21.696 ± 1.233
NHD–M	9.276 ± .074	1.435 ± .053	15.464 ± .579
NHH–J	10.029 ± .078	1.678 ± .055	16.731 ± .567
NHH–M	9.889 ± .041	1.595 ± .029	16.132 ± .303
NHHH–J	8.649 ± .033	1.388 ± .023	16.049 ± .275
NHHH–M	8.686 ± .023	1.300 ± .017	14.792 ± .194
NHDD–J	9.597 ± .032	1.416 ± .023	14.753 ± .244
NHDD–M	9.465 ± .028	1.437 ± .020	15.187 ± .213
NDH–D	9.040 ± .057	1.123 ± .040	12.426 ± .454
NDH–E	8.849 ± .048	1.134 ± .034	12.812 ± .387
NDD–D	8.955 ± .116	1.618 ± .082	18.073 ± .948
NDD–E	8.649 ± .109	1.216 ± .077	14.055 ± .905
NDDD–D	8.628 ± .041	1.184 ± .029	13.719 ± .339
NDDD–E	8.739 ± .048	1.379 ± .034	15.781 ± .398
NDHH–D	8.607 ± .037	1.254 ± .026	14.565 ± .313
NDHH–E	8.732 ± .038	1.188 ± .027	13.610 ± .311
USS–I	14.215 ± .056	2.730 ± .040	19.208 ± .288
USS–K	14.911 ± .045	2.226 ± .032	14.930 ± .220
FSS–I	6.860 ± .027	1.057 ± .019	15.407 ± .284
FSS–L	6.947 ± .019	.950 ± .013	13.678 ± .193
FSH–C	8.521 ± .030	.959 ± .021	11.259 ± .250
FSD–C	7.232 ± .044	.996 ± .031	13.769 ± .439
FSHH–C	7.243 ± .025	1.083 ± .018	14.955 ± .248
FSDD–C	7.378 ± .028	1.094 ± .020	14.822 ± .277
GGH–F	18.841 ± .073	2.629 ± .052	13.953 ± .278
GGH–G	18.796 ± .119	2.446 ± .084	13.016 ± .457
GGH–K	18.790 ± .085	2.622 ± .060	13.956 ± .325
GGH$_2$–F	18.635 ± .078	2.396 ± .055	12.855 ± .302
GGH$_2$–G	18.440 ± .117	2.720 ± .082	14.749 ± .456
GGH$_2$–K	19.170 ± .096	2.633 ± .068	13.734 ± .361
GGD–F	15.140 ± .108	2.377 ± .076	15.699 ± .515
GGD–G	14.942 ± .160	2.601 ± .113	17.406 ± .864
GGD–K	14.776 ± .106	2.423 ± .075	16.398 ± .521
GGD$_2$–F	16.354 ± .079	2.347 ± .056	14.349 ± .349
GGD$_2$–G	16.275 ± .087	2.253 ± .062	13.844 ± .387
GGD$_2$–K	16.847 ± .086	2.593 ± .061	15.391 ± .370
GGHH–F	18.459 ± .097	2.511 ± .069	13.602 ± .380
GGHH–G	18.319 ± .149	2.381 ± .105	12.997 ± .585
GGHH–K	18.298 ± .096	2.439 ± .068	13.328 ± .379
GGDD–F	16.379 ± .087	2.311 ± .061	14.106 ± .382
GGDD–G	16.681 ± .150	2.588 ± .106	15.513 ± .652
GGDD–K	16.106 ± .083	2.243 ± .059	13.929 ± .373
LL–A	13.522 ± .193	2.380 ± .137	17.604 ± 1.042
LL–B	13.115 ± .247	1.866 ± .175	14.224 ± 1.357
LLS–A	13.759 ± .109	3.097 ± .077	22.511 ± .590
LLS–B	13.280 ± .140	2.943 ± .099	22.157 ± .783
LLS–H	13.772 ± .042	2.902 ± .030	21.068 ± .223
LLS–K	13.638 ± .116	2.671 ± .082	19.587 ± .622
W–K	19.329 ± .108	3.753 ± .076	19.417 ± .410
GGS–L	19.972 ± .141	2.799 ± .100	14.013 ± .509

TABLE VIII

PHYSICAL CONSTANTS FOR SEEDS MORE OR LESS ABNORMAL IN GERMINATION

Series	Mean and Probable Error	Standard Deviation and Probable Error	Coefficient of Variation and Probable Error
NHD–J	8.906 ± .159	1.714 ± .112	19.251 ± 1.307
NHD–M	9.325 ± .933	1.305 ± .660	13.993 ± .901
NHH–J	9.603 ± .109	1.229 ± .077	12.794 ± .814
NHH–M	10.047 ± .080	1.445 ± .057	14.387 ± .576
NHHH–J	8.537 ± .155	1.469 ± .109	17.209 ± 1.319
NHHH–M	8.679 ± .141	1.526 ± .200	17.578 ± 1.187
NHDD–J	9.019 ± .150	1.633 ± .106	18.107 ± 1.213
NHDD–M	9.175 ± .115	1.284 ± .811	13.933 ± .901
NDH–D	9.045 ± .076	1.198 ± .054	13.244 ± .607
NDH–E	8.984 ± .078	1.296 ± .055	14.429 ± .626
NDD–D	8.667 ± .151	1.601 ± .107	18.477 ± 1.275
NDD–E	9.105 ± .151	1.382 ± .107	15.180 ± 1.202
NDDD–D	8.649 ± .111	1.245 ± .079	14.395 ± .928
NDDD–E	8.333 ± .186	1.509 ± .131	18.111 ± 1.628
NDHH–D	8.444 ± .148	1.740 ± .105	20.609 ± 1.290
NDHH–E	8.902 ± .128	1.215 ± .091	13.647 ± 1.035
USS–I	14.019 ± .302	3.257 ± .213	23.236 ± 1.602
USS–K	14.766 ± .167	2.403 ± .118	16.275 ± .821
FSS–I	6.507 ± .071	.860 ± .050	13.219 ± .784
FSS–L	7.110 ± .076	1.184 ± .054	16.647 ± .778
FSH–C	8.896 ± .068	1.123 ± .048	12.628 ± .545
FSD–C	6.892 ± .077	.859 ± .055	12.468 ± .807
FSHH–C	7.115 ± .100	1.068 ± .071	15.004 ± 1.014
FSDD–C	7.170 ± .104	1.125 ± .074	14.792 ± 1.051
GGH–F	19.279 ± .130	2.310 ± .092	11.980 ± .485
GGH–G	19.238 ± .227	2.674 ± .161	13.899 ± .851
GGH–K	19.382 ± .197	2.759 ± .140	14.237 ± .734
GGH$_2$–F	20.400 ± .317	2.349 ± .224	11.514 ± 1.113
GGH$_2$–G	19.833 ± .375	2.721 ± .265	13.718 ± 1.360
GGH$_2$–K	18.333 ± .933	3.389 ± .660	18.485 ± 3.720
GGD–F	14.295 ± .123	2.007 ± .087	14.041 ± .618
GGD–G	14.950 ± .150	2.228 ± .106	14.903 ± .727
GGD–K	14.491 ± .085	1.896 ± .060	13.083 ± .420
GGD$_2$–F	15.321 ± .295	2.316 ± .209	15.114 ± 1.393
GGD$_2$–G	16.464 ± .422	3.311 ± .298	20.109 ± 1.884
GGD$_2$–K	16.750 ± .344	2.281 ± .243	13.619 ± 1.479
GGHH–F	18.947 ± .420	2.711 ± .297	14.308 ± 1.597
GGHH–G	19.417 ± .544	2.795 ± .385	14.396 ± 2.023
GGHH–K	18.556 ± .872	3.878 ± .617	20.897 ± 3.464
GGDD–F	16.363 ± .541	2.660 ± .383	16.259 ± 2.399
GGDD–G	16.875 ± .339	1.423 ± .240	8.435 ± 1..32
GGDD–K	15.667 ± .669	3.434 ± .473	21.921 ± 3.160
LL–A	14.932 ± .183	2.080 ± .129	13.927 ± .881
LL–B	14.152 ± .223	1.899 ± .158	13.420 ± 1.134
LLS–A	17.153 ± .209	3.550 ± .148	20.693 ± .898
LLS–B	16.473 ± .303	3.327 ± .214	20.194 ± 1.351
LLS–H	16.652 ± .080	3.538 ± .057	21.244 ± .355
LLS–K	16.244 ± .185	3.327 ± .131	20.482 ± .839
W–K	20.683 ± .384	4.410 ± .272	21.321 ± 1.371
GGS–L	19.900 ± .110	2.594 ± .078	13.038 ± .401

TABLE IX

PHYSICAL CONSTANTS FOR SEEDS FAILING TO GERMINATE

Series	Mean and Probable Error	Standard Deviation and Probable Error	Coefficient of Variation and Probable Error
NHD–J	8.594 ± .026	1.413 ± .019	16.443 ± .223
NHD–M	8.659 ± .023	1.357 ± .016	15.675 ± .194
NHH–J	9.864 ± .027	1.397 ± .019	14.166 ± .195
NHH–M	9.905 ± .024	1.379 ± .017	13.917 ± .175
NHHH–J	8.654 ± .037	1.363 ± .026	15.752 ± .308
NHHH–M	8.491 ± .069	1.505 ± .049	17.723 ± .590
NHDD–J	9.351 ± .046	1.476 ± .033	15.781 ± .357
NHDD–M	8.821 ± .066	1.401 ± .046	15.878 ± .540
NDH–D	8.624 ± .038	1.239 ± .027	14.367 ± .316
NDH–E	8.804 ± .032	1.237 ± .022	14.050 ± .259
NDD–D	8.411 ± .038	1.314 ± .027	15.617 ± .331
NDD–E	8.444 ± .039	1.307 ± .028	15.484 ± .334
NDDD–D	8.746 ± .058	1.336 ± .041	15.275 ± .477
NDDD–E	8.531 ± .059	1.322 ± .042	15.499 ± .501
NDHH–D	8.564 ± .045	1.237 ± .032	14.444 ± .383
NDHH–E	8.701 ± .053	1.211 ± .384	13.917 ± .450
USS–I	14.435 ± .085	2.664 ± .060	18.451 ± .430
USS–K	15.092 ± .101	2.453 ± .071	16.251 ± .483
FSS–I	6.422 ± .025	1.066 ± .018	16.606 ± .285
FSS–L	6.693 ± .026	1.147 ± .019	17.134 ± .286
FSH–C	8.565 ± .035	1.073 ± .025	12.521 ± .297
FSD–C	6.779 ± .034	1.168 ± .024	17.235 ± .367
FSHH–C	7.331 ± .042	1.123 ± .030	15.317 ± .414
FSDD–C	7.018 ± .042	1.143 ± .030	16.284 ± .438
GGH–F	19.584 ± .129	2.979 ± .091	15.210 ± .476
GGH–G	19.194 ± .119	2.934 ± .084	15.285 ± .447
GGH–K	19.330 ± .131	2.729 ± .093	14.116 ± .489
GGH$_2$*–F*	18.597 ± .235	2.745 ± .116	14.761 ± .913
GGH$_2$*–G*	18.718 ± .184	2.583 ± .134	13.798 ± .727
GGH$_2$*–K*	19.328 ± .238	2.688 ± .168	13.906 ± .887
GGD–F	14.286 ± .172	2.334 ± .121	16.335 ± .872
GGD–G	14.500 ± .128	2.598 ± .090	17.920 ± .643
GGD–K	14.690 ± .106	2.141 ± .075	14.578 ± .519
GGD$_2$*–F*	15.400 ± .216	2.262 ± .153	14.688 ± 1.012
GGD$_2$*–G*	15.827 ± .218	3.483 ± .154	22.005 ± 1.020
GGD$_2$*–K*	16.302 ± .223	2.628 ± .158	16.122 ± .994
GGHH–F	18.321 ± .221	2.451 ± .156	13.378 ± .868
GGHH–G	18.978 ± .270	2.687 ± .191	14.157 ± 1.027
GGHH–K	19.341 ± .294	2.787 ± .208	14.412 ± 1.095
GGDD–F	15.103 ± .325	2.591 ± .229	17.154 ± 1.563
GGDD–G	16.019 ± .225	2.406 ± .159	15.020 ± 1.016
GGDD–K	15.822 ± .293	2.918 ± .208	18.442 ± 1.355
LL–A	14.356 ± .070	2.704 ± .050	18.832 ± .467
LL–B	14.154 ± .070	2.280 ± .049	16.106 ± .357
LLS–A	14.571 ± .266	4.036 ± .188	27.698 ± 1.384
LLS–B	15.036 ± .142	3.794 ± .100	25.233 ± .706
LLS–H	16.480 ± .117	4.169 ± .082	25.295 ± .531
LLS–K	16.362 ± .376	4.240 ± .266	25.914 ± 1.728
W–K	19.092 ± .282	4.140 ± .199	21.683 ± 1.093
GGS–L	20.350 ± .078	2.867 ± .055	14.088 ± .278

ment, it is necessary to note that, except for the coefficients of variation, these constants are in absolute values. Clearly enough a difference of .254 unit in mean or of .197 in S.D. for White Flageolet beans with an average weight of 6.755 units and a S.D. of 1.071 units is not comparable with a difference of the same absolute amount in Golden Wax or Burpee's Stringless with a mean weight of, say, 18.401 and a scatter in weight of 2.544 units.

TABLE X[13]

PHYSICAL CONSTANTS FOR GENERAL POPULATION

Series	N	Mean and Probable Error	Standard Deviation and Probable Error	Coefficient of Variation and Probable Error
NHD.....	6,630	8.529 ± .012	1.458 ± .009	17.099 ± .103
NHH.....	7,334	9.774 ± .011	1.421 ± .008	14.537 ± .082
NHHH...	5,601	8.609 ± .012	1.338 ± .009	15.543 ± .101
NHDD....	5,029	9.417 ± .014	1.484 ± .099	15.763 ± .109
NDH.....	3,227	8.852 ± .015	1.555 ± .011	14.089 ± .121
NDD.....	2,362	8.487 ± .019	1.377 ± .014	16.218 ± .163
NDDD....	1,946	8.649 ± .020	1.315 ± .014	15.210 ± .168
NDHH ...	2,433	8.604 ± .017	1.252 ± .012	14.549 ± .144
USS.....	3,271	14.640 ± .030	2.519 ± .021	17.205 ± .148
FSS.....	3,740	6.755 ± .012	1.071 ± .008	15.854 ± .126
FSH.....	2,122	8.516 ± .016	1.092 ± .011	12.826 ± .135
FSD.....	1,989	6.956 ± .016	1.034 ± .011	14.858 ± .161
FSHH....	1,788	7.225 ± .017	1.080 ± .012	14.953 ± .172
FSDD....	1,643	7.213 ± .019	1.127 ± .013	15.623 ± .188
GGH.....	2,828	18.919 ± .034	2.674 ± .024	14.131 ± .177
GGH_2.....	1,284	18.799 ± .049	2.608 ± .034	13.873 ± .188
GGD.....	2,140	14.972 ± .036	2.498 ± .026	16.681 ± .193
GGD_2.....	1,419	16.379 ± .046	2.577 ± .033	15.732 ± .204
GGHH....	1,329	18.401 ± .047	2.544 ± .033	13.824 ± .184
GGDD....	1,093	16.298 ± .048	2.395 ± .044	14.700 ± .216
LL.....	1,070	14.206 ± .050	2.443 ± .036	17.197 ± .260
LLS.....	5,305	14.826 ± .033	3.570 ± .023	24.077 ± .167
W.....	707	19.412 ± .099	3.888 ± .070	20.032 ± .374
GGS.....	1,039	20.176 ± .059	2.800 ± .041	13.876 ± .209

[13] These constants are, except for the *LL*, *LLS* and *GGS* series, calculated directly from the data tabled for the general populations. In the case of the *LL* series the seeds were already a selected class—the heavier and lighter having been drawn for the planting giving rise to *LLS* plants. Hence in this case the constants were based on the summed seriations for the seeds failing, producing abnormal seedlings and producing normal seedlings in the two lots. They will differ somewhat from those of the whole population of seeds weighed. In the *LLS* and *GGS* series the tables for the general population were not yet prepared, hence the seriations of the seeds of classes (A)–(C) were summed for the various experiments and served as the basis for the general population constant.

Hence these absolute differences must, for the sake of convenience and of strict comparability, be reduced to relative terms. The best way of doing this is to express them in percentages of the general population values for the same constant, where "general population" means the whole mass of the particular strain and series of seeds from which the seeds for the individual experiments were drawn.

In the discussion of the whole series of experiments both absolute and relative values will be taken into account. In the preparation of the diagrams for differences in mean and S.D. the relative (percentage) values only will be used.

Table X gives the physical constants for the general populations, and the numbers of seeds upon which they are based.

I now turn to the various comparisons. It would be desirable to place before the reader the individual differences and their probable errors, but since these number 750 their publication is precluded by lack of space, and the small summary tables must suffice. All these differences may, of course, be derived by the reader caring to check the arithmetic from the tables of fundamental constants.

(To be concluded)

RECIPROCAL CROSSES BETWEEN REEVES'S PHEASANT AND THE COMMON RING-NECK PHEASANT PRODUCING UNLIKE HYBRIDS

MANY sex-linked characters have been described in birds (fowls, pigeons, canaries and doves). The pheasant hybrids to be described, however, show merely a different appearance of male sexual plumage characters in the F_1 hybrids of a reciprocal cross between Reeves's pheasant and the common ring-neck pheasant (*P. torquatus*). These hybrids are sterile, and therefore the experiment ends with the first cross, although Cronau[1] stated that the offspring from a Reeves's cock and common pheasant hen were occasionally fertile. Poll,[2] however, who studied the spermatogenesis of numerous pheasant crosses, found the hybrids between Reeves's and the common pheasants and between Reeves's and Sommerings's pheasants always sterile.

The Reeves's pheasant was originally given generic recognition by Wagler under the name *Syrmaticus reevesi*. This distinction it certainly deserves, although later writers have often placed it under *Phasianus*. The ring-neck pheasant, so called, refers to the common stock pheasant which is now practically pure *torquatus*.

In the fall of 1911 two hens were mated as follows: Pen D contained a ♂ Reeves's with two ring-neck hens; pen H a ♂ ring-neck with two Reeves's hens. These were all birds of the season. The Reeves's were from the same clutch of eggs from a single pair, and the ring-necks from a strain of which large numbers have been bred on the farm. The Reeves's never, to my knowledge, shows any variation of plumage in captivity. The strain of ring-necks is practically constant, though the white neck ring sometimes differs in its width.

It is therefore fair to suppose that the somatic difference of the hybrids to be described is a constant feature, although from pen D only two males were reared to maturity, and from pen H only four. The six birds, however, immediately fall into two classes. They have all the appearance of two well-marked species. Hens were reared only from pen H.

[1] Cronau, C., *Zool. Garten.*, 1899, p. 99.

[2] Poll, H., *Gesellschaft Natur.-Freunde,* 1908, p. 127.

A large number of eggs from these two pens was set, but from pen D only five chicks were hatched; from pen H, ten. These two lots of chicks were noted as differing both in down and in first plumage in the following way: those with the Reeves's father and ring-neck mother, pen D, were lighter-colored than the birds of the reciprocal cross. No detailed observations were made. On maturity this same difference was found to hold. On comparing the adult specimens dorsal side up, there is at once seen to be a constant difference involving all the feather regions. In general, it may be said that in cross D the Reeves's father transmitted to his hybrid offspring more of his own characters than the female Reeves's transmitted to her offspring in cross H. This is especially shown in the almost pure Reeves's head pattern of cross D, and in the general lighter tone of the whole upper parts and flanks.

On the other hand, the stronger tail barring of Reeves's pheasant, as contrasted with the ring-neck, has been transmitted to cross H by the Reeves's hen, and has not been carried to the same extent by the male Reeves's in the other cross.

The plate shows the difference, and needs no explanation. The other differences are briefly as follows:

Cross D, feathers of mantle with reduced and irregular black band.

H, feathers of mantle with broad black band.

D, feathers of mantle tending to sub-terminal bar of buckthorn brown (Ridgway, 1912).

H, brown bar absent.

D, general color of mantle more tawny and less dark than in H. Back and rump much lighter than in H, with also an entirely different feather pattern. Upper tail coverts lighter in D than in H. Barring of tail reduced in D to basal third and not heavy. In H, heavy barring of whole tail, becoming blotchy and obscured towards terminal third.

Scapulars, greater and lesser wing coverts, and even primary quills different in the two crosses; and tending to more rich browns and larger light areas in D than in H. First primary with larger and more distinct light bars on inner web in D than in H.

Flanks lighter and with tawny sub-terminal bars in D, which are not present in H.

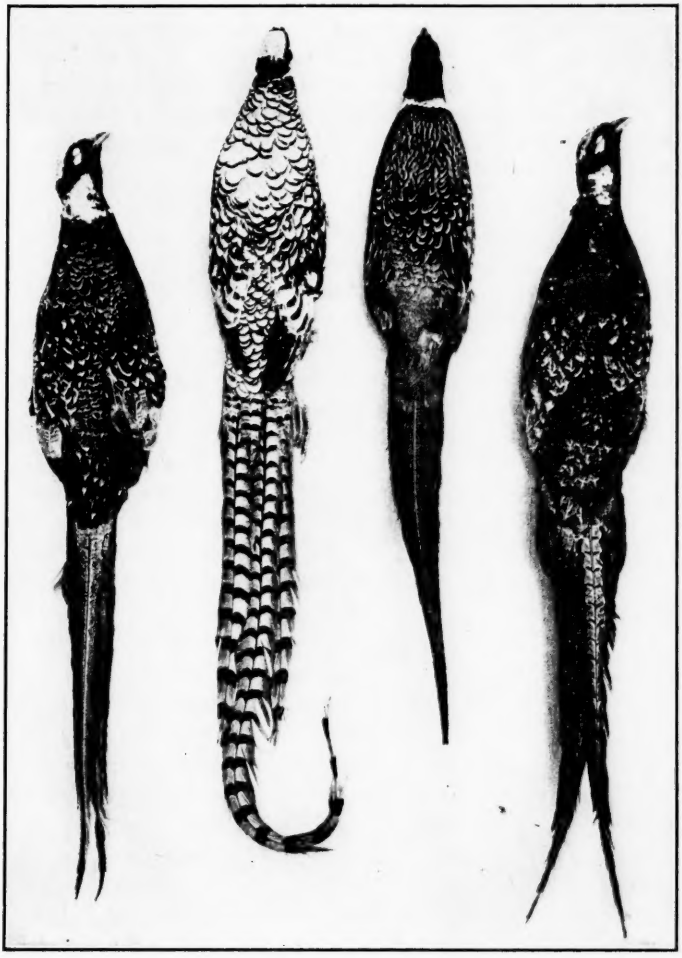

FIG. 1. Male hybrid from a mating of a male Reeves's with a female ring-neck pheasant.
FIG. 2. Male Reeves's pheasant.
FIG. 3. Male ring-neck pheasant.
FIG. 4. Male hybrid from a mating of a male ring-neck with a female Reeves's pheasant.

Breast and lower throat slightly darker in H than in D, but very similar. Rest of lower parts about the same in both crosses.

Three hen birds were reared from pen H. They all showed strong tail barring and other well-marked Reeves's characters.

The females of the two species involved are quite different, and it is therefore to be regretted that there are no specimens from both crosses for comparison.

SUMMARY

That this somatic difference between reciprocal crosses in other pheasants is not always present, is shown by the uniform F_1 generation in the two crosses, Amherst $\times$ Gold, of the genus *Chrysolophus*, bred by myself. In the work of Professor Allesandro Ghigi and Mrs. Haig-Thomas on pheasants no reciprocal crosses have apparently been made.

The significance of the present case is not clear, and it is desired simply to put it on record. Further work is necessary to prove that reciprocal crosses between Reeves and the true pheasants always give different results.

It is interesting to note that the differences which have been described are rather subtle ones and quantitative rather than qualitative.

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